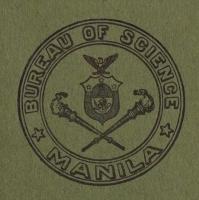
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#### A CONTRIBUTION TO THE CHEMICAL STUDY OF THE BLOOD OF PHILIPPINE CARABAOS

By ARCADIO C. GONZAGA

Of the College of Veterinary Science, University of the Philippines, Manila

Data on the physiology of the Philippine carabao (Bubalus bubalis L.) are very meager, especially those relating to its blood chemistry. A survey of the literature has shown that, so far, the only available report on some blood constituents of this animal is that of Posa, (8) who made observations on five males and five females.

The purpose of the present article is to contribute more data on the physiology of the blood of the Philippine carabao.

#### MATERIALS AND METHODS

The observations were made on twenty carabaos, fourteen males and six females, 4 to 9 years old, and, as far as could be ascertained, free from disease.

The blood samples were drawn from the jugular vein and collected in clean vials. Where uncoagulated whole blood was needed, potassium oxalate was used as anticoagulant; where serum was necessary, the blood was allowed to coagulate and the serum separated from the clot after twenty-four hours, centrifuged to remove suspended debris, and kept in a refrigerator until used. Blood filtrates prepared according to Folin's unlaked blood method(3) were used in the determination of blood sugar, total nonprotein nitrogen, urea nitrogen, and chlorides. When not in use the filtrates were kept at refrigerator temperature.

The blood sugar was determined according to the colorimetric copper reduction method of Folin; (2) the total nonprotein nitro-

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TABLE 1.—Constituents of the blood of carabaos.

From Sugar. Total Trogen. Introgen. Introdeced. Introduced Intr	
Sugar.         Total nitrogen.         Calcium.         Inorganic phose-as NaCl. phases.	9
40.16         18.80         21.80         10.50         7.35         446.00         51.81           49.51         25.55         15.72         10.00         8.90         485.00         46.51           89.22         22.83         13.49         9.50         4.85         495.00         42.19           89.84         22.11         15.63         10.00         4.10         480.00         42.19           45.87         24.63         13.44         9.50         4.16         480.00         50.76           44.84         22.11         15.63         10.00         4.16         480.00         50.76           44.84         19.29         18.73         9.50         6.45         465.00         51.28           46.30         20.63         18.72         10.50         4.18         470.00         41.49           41.84         20.11         16.70         10.50         4.18         470.00         41.49           41.84         20.11         16.75         10.00         5.95         496.00         41.49           41.84         20.11         16.76         9.50         6.45         400.00         41.67           41.84         20.11         1	Sex. Age.
40.16         18.80         21.80         10.60         7.35         446.00         51.81         15.47           56.25         25.56         15.72         10.00         8.90         485.00         46.51         13.83           56.26         25.36         15.72         10.00         4.35         465.00         46.51         13.83           89.22         28.73         12.44         9.50         4.35         465.00         42.19         12.59           44.45         22.11         15.63         10.00         4.16         465.00         50.76         15.15           44.45         20.21         16.63         10.00         4.16         465.00         51.28         15.15           44.85         18.72         18.73         9.50         4.18         465.00         41.49         16.39           46.30         18.72         12.66         10.50         4.18         466.00         41.49         12.39           40.98         18.72         12.66         10.50         5.96         456.00         42.56         12.49           40.98         18.72         12.68         10.50         5.96         456.00         41.67         11.39	Years.
49. 51         25. 67         15.72         10.00         8.90         485.00         46.51         13.88           89. 22         28.78         13. 50         10.50         4.86         465.00         48.54         14.49           89. 22         28.78         12.44         9.50         4.16         480.00         48.54         14.49           46. 87         22.11         15.63         10.00         4.16         480.00         50.78         15.15           46. 87         22.11         15.63         10.00         4.16         480.00         50.78         15.15           46. 87         20.42         18.12         10.00         4.16         490.00         50.78         15.15           46. 87         20.42         18.12         10.00         6.45         450.00         51.28         15.30           46. 80         19.26         14.62         10.00         5.96         456.00         42.56         12.39           40. 98         18.72         10.00         5.96         456.00         42.65         11.39           40. 98         18.72         10.00         5.96         456.00         42.65         11.39           40. 80         18.7	100
55.26         25.36         13.50         4.85         450.00         48.54         14.49           39.42         28.78         12.44         9.50         4.36         46.60         48.64         12.64           45.87         22.61         15.46         9.50         4.16         495.00         50.76         15.15           45.87         22.63         14.49         9.50         4.16         495.00         50.76         15.15           44.45         20.42         18.72         10.50         4.16         495.00         51.28         15.39           46.30         20.62         15.70         10.50         4.18         470.00         51.65         15.39           40.98         18.72         12.66         10.50         4.18         470.00         42.55         12.70           40.98         18.72         12.66         10.50         4.18         470.00         42.56         15.70           41.84         19.29         14.62         10.00         5.96         478.00         42.56         12.70           41.49         10.10         0.00         5.96         478.00         42.16         11.99           41.49         10.10 <t< td=""><th>M</th></t<>	M
39.22         28.78         12.44         9.50         4.36         465.00         42.19         12.59           45.87         22.11         15.63         10.00         4.16         480.00         50.76         15.15           44.45         20.42         13.78         9.50         4.16         465.00         48.08         14.35           41.32         18.95         18.12         10.50         4.18         465.00         41.49         15.30           46.30         20.63         15.70         10.50         4.18         470.00         41.49         12.30           46.30         18.72         12.56         10.00         5.66         465.00         41.49         12.70           41.84         19.29         16.70         7.15         495.00         40.16         11.39           41.49         19.10         10.00         5.96         496.00         41.44         11.99           41.49         19.10         10.00         5.96         496.00         41.47         11.99           41.49         19.10         10.00         5.96         496.00         41.47         11.99           44.64         18.44         18.34         10.90	M
48, 87         22.11         15.63         10.00         4.10         480.00         50.76         15.15           44, 45         20.42         13.78         3.50         6.45         465.00         51.28         15.15           44, 45         20.42         18.78         3.50         6.45         465.00         51.26         15.39           46, 30         18.78         10.50         4.16         465.00         41.49         12.39           46, 30         19.29         14.52         10.50         4.18         46.00         41.49         12.39           40, 98         18.72         12.56         10.50         5.96         495.00         41.67         12.39           40, 98         18.72         12.56         10.00         5.96         495.00         41.67         11.99           40, 98         18.74         10.00         5.95         490.00         42.84         13.99           50, 25         22.66         15.11         9.50         7.89         490.00         42.84         13.59           44, 64         18.46         12.00         6.37         490.00         42.84         13.69           44, 64         18.46         10.00 <th>M</th>	M
45.87         24.68         14.49         9.50         4.16         495.00         51.28         15.31           44.45         20.42         18.78         9.50         6.45         465.00         51.28         16.31           41.32         18.96         18.72         10.50         4.55         479.00         51.28         14.35           46.30         20.63         15.70         10.50         4.56         405.00         41.49         12.89           40.38         18.72         12.56         10.00         5.66         465.00         42.56         12.49           41.49         19.16         12.98         10.00         5.96         495.00         41.67         12.44           41.49         19.16         12.98         10.00         5.95         496.00         42.67         12.44           41.49         19.66         16.01         6.95         490.00         42.67         12.44           44.64         18.46         12.67         10.00         4.96         490.00         42.84         18.39           46.30         19.08         17.34         10.50         6.96         490.00         42.65         12.47           46.31	M
44,45         20.42         18.78         3.50         6.45         46.50         48.08         14.35           46,38         18.95         18.12         10.50         4.15         4.65         10.50         4.18         470.00         6.15         15.39           46,38         20.63         14.52         10.00         5.66         466.00         42.65         15.70           40,98         18.72         12.56         10.00         5.96         466.00         42.65         12.70           41.84         19.16         16.76         9.60         7.15         486.00         41.67         12.44           41.49         19.16         16.76         9.60         7.89         490.00         42.02         12.54           44.64         18.46         16.00         6.95         490.00         42.02         12.54           46.30         19.08         17.34         10.00         6.95         490.00         49.26         14.70           46.30         18.14         16.31         10.00         6.95         490.00         40.32         12.04           46.51         18.14         16.21         10.00         6.95         490.00         40.32	
41.32         18.15         18.12         10.50         4.55         479.00         51.55         15.39           46.30         20.63         16.70         10.50         4.18         470.00         51.55         15.39           46.38         18.72         12.56         10.00         5.66         465.00         40.16         11.39           41.84         20.11         16.76         9.50         7.15         495.00         40.16         11.39           41.84         19.16         12.98         10.00         5.95         495.00         40.16         11.39           50.25         22.66         15.11         9.50         7.89         490.00         49.26         14.70           46.30         18.46         12.67         10.00         6.36         490.00         49.26         14.70           46.30         18.14         16.31         10.50         4.36         465.00         46.87         13.54           46.51         20.04         13.38         10.50         8.00         475.00         40.32         12.04           46.51         21.16         10.50         8.00         446.00         41.15         12.28           44.00	
46.30         20.65         15.70         10.50         4.18         470.00         41.49         12.39           46.84         19.29         14.52         10.00         6.56         466.00         42.55         12.95           40.98         18.72         12.56         10.50         6.96         466.00         41.67         12.94           41.49         19.16         12.98         10.50         7.16         486.00         41.67         12.44           41.49         19.16         12.98         10.00         6.96         49.60         44.84         13.54           44.64         18.46         12.67         10.00         4.96         490.00         49.86         14.70           46.30         19.08         17.34         10.50         6.87         496.00         45.87         15.63           41.15         22.39         14.23         9.50         4.96         465.00         46.87         15.04           46.51         21.16         17.80         10.50         8.00         476.00         41.15         12.38           44.00         21.23         12.44         9.50         3.90         446.00         41.15         12.30	0
44.84         19.29         14.52         10.00         5.66         456.00         42.55         12.70           40.38         18.72         12.56         10.50         6.95         495.00         40.16         11.39           41.84         20.11         16.75         9.50         7.15         486.00         44.84         11.39           41.84         20.11         16.75         9.50         7.15         486.00         44.84         18.39           50.25         22.65         15.11         9.50         7.89         490.00         42.02         12.54           46.80         19.08         17.34         10.00         6.37         490.00         42.26         14.70           46.81         18.44         16.21         10.00         6.37         490.00         49.26         15.31           46.02         18.14         16.31         10.00         6.37         496.00         40.32         12.04           46.51         22.39         14.23         9.50         4.96         496.00         40.32         12.04           46.51         21.16         10.50         8.00         476.00         41.15         12.28           44.00         <	-
40.98         18.72         12.56         10.50         6.96         496.00         40.16         11.99           41.84         20.11         16.75         9.50         7.15         486.00         41.67         12.44           41.49         19.11         12.98         10.00         5.96         478.00         42.02         12.44           44.64         18.46         12.67         10.00         5.96         490.00         42.02         12.54           46.30         18.46         12.67         10.00         6.96         490.00         45.26         14.70           40.32         18.14         16.31         10.00         6.37         456.00         45.26         14.70           40.32         18.14         16.21         10.00         6.37         456.00         40.32         12.04           40.32         18.14         16.23         10.50         6.65         456.00         40.32         12.04           46.51         21.16         17.80         10.50         8.00         446.00         41.15         12.28           46.51         21.23         18.14         9.50         8.00         466.00         41.15         12.29	
41.84         20.11         16.76         9.50         7.15         486.00         41.67         12.44           41.49         19.16         12.98         10.00         6.95         478.00         44.84         18.35           50.25         22.65         15.11         9.50         7.89         490.00         49.26         18.38           46.30         19.08         17.34         10.00         6.37         495.00         45.87         18.70           46.30         18.14         16.31         10.00         6.37         495.00         45.87         15.81           41.15         22.39         14.23         9.50         4.96         465.00         61.28         15.81           46.51         20.04         13.38         10.50         8.00         475.00         41.15         12.28           44.00         21.23         10.50         8.00         476.00         41.15         12.28           55.25         28.78         21.80         40.00         40.16         11.99           44.40         21.23         10.50         8.00         466.00         41.15         12.28           55.25         28.74         10.00         8.00         <	1
41.49         19.16         12.98         10.00         6.95         478.0         44.84         18.39           44.64         18.46         12.67         10.00         4.96         400.00         42.02         12.64           46.80         19.08         17.34         10.00         4.96         400.00         45.87         13.69           40.32         18.14         16.31         10.00         6.37         496.00         45.87         13.69           41.15         22.39         14.23         9.50         4.95         465.00         61.28         15.04           46.51         20.04         13.38         10.50         6.65         495.00         41.15         12.04           46.51         21.16         17.80         10.50         8.00         475.00         41.15         12.28           44.00         21.23         110.05         8.00         476.00         41.15         12.28           55.25         28.78         12.80         10.50         8.00         466.00         40.16         11.99           44.42         21.80         10.60         8.00         466.00         51.81         16.47           48.01         20.04	
60.25         22.66         15.11         9.50         7.89         490.00         42.02         12.64           46.64         18.46         12.67         10.00         4.96         490.00         42.02         12.54           40.32         18.14         15.31         10.50         7.15         490.00         49.27         13.69           40.32         18.14         15.31         10.00         6.37         490.00         49.28         14.31           41.15         22.39         14.23         9.50         4.96         496.00         50.12         15.31           46.51         21.16         17.80         10.50         6.65         496.00         41.15         12.28           44.00         21.23         15.19         10.05         8.00         476.00         41.15         12.28           55.25         28.78         21.80         10.06         8.00         446.00         40.16         11.39           55.25         28.74         12.80         10.07         6.63         481.71         46.88         13.70           44.4.22         21.74         12.80         10.07         6.18         470.17         46.69         13.91	
44.64         18.46         12.67         10.00         4.90         49.25         14.70         18.46           46.80         19.08         17.34         10.50         7.15         485.00         45.77         13.79           40.32         18.14         16.31         10.00         6.37         456.00         40.32         12.04           41.15         22.39         14.23         9.50         4.96         456.00         60.51         15.04           46.51         21.16         17.80         10.50         6.65         456.00         41.15         12.28           44.00         21.23         15.19         10.05         8.00         446.00         41.15         12.28           55.25         28.78         21.80         10.50         8.00         496.00         51.81         15.47           44.4.22         21.74         12.80         10.07         6.63         481.71         46.88         13.70           43.01         20.04         16.52         10.00         6.18         470.17         46.69         18.31	
46.30         13.06         17.34         10.00         6.37         495.00         5.27         495.00         5.37         495.00         5.31         40.32         15.31         40.32         12.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32         13.04         40.32	
40.52         20.04         11.53         14.23         9.50         4.95         450.00         40.22         12.04           49.68         20.04         13.38         10.50         6.66         495.00         60.51         15.03           46.51         21.16         17.80         10.50         8.00         475.00         41.15         12.28           44.00         21.23         15.19         10.05         5.78         478.25         46.09         13.76           55.25         28.78         12.44         9.50         3.90         446.00         40.16         11.99           44.42         21.80         10.50         8.00         450.00         51.81         16.47           48.01         20.04         16.52         10.07         6.63         491.71         46.68         13.70	
39.68         20.04         13.38         10.50         6.65         495.00         60.51         15.08           46.51         21.16         17.80         10.50         8.00         475.00         41.15         12.28           44.00         21.23         15.19         10.05         5.78         478.25         46.09         13.76           55.25         28.78         12.40         9.50         3.90         446.00         40.16         11.99           44.42         21.74         12.80         10.07         5.63         481.71         45.69         13.70           48.01         20.04         16.52         10.00         6.13         470.17         46.69         13.91	4 6
46.51         21.16         17.80         10.50         8.00         475.00         41.15         12.28           44.00         21.23         15.19         10.05         5.78         478.25         46.09         18.76           39.22         18.14         12.44         9.50         8.00         446.00         40.16         11.99           55.25         28.78         21.80         10.50         8.00         495.00         51.81         15.47           44.42         21.74         12.80         10.07         6.63         481.71         46.89         18.70           48.01         20.04         16.52         10.00         6.18         470.17         46.69         18.91	
21.23         15.19         10.05         5.78         478.25         46.09         18.76           18.14         12.44         9.50         8.00         446.00         40.16         11.99           28.78         21.80         10.50         8.00         495.00         51.81         15.47           21.74         12.80         10.07         5.63         481.71         46.88         18.70           20.04         16.52         10.00         6.18         470.17         46.59         13.91	-
18.14         12.44         9.50         8.95         446.00         40.16         11.39           28.78         21.80         10.50         8.00         495.00         51.81         15.47           21.74         12.80         10.07         5.63         481.71         45.88         13.70           20.04         16.52         10.00         6.13         470.17         46.59         13.91	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
28.78         21.80         10.50         8.00         495.00         51.81         15.47           21.74         12.80         10.07         5.63         481.71         46.88         13.70           20.04         16.52         10.00         6.13         470.17         46.59         13.91	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
21.74 12.80 10.07 5 68 481.71 46.88 13.70 20.04 16.52 10.00 6.13 470.17 46.59 13.91	6 1 6
20.04 16.52 10.00 6.18 470.17 46.59 13.91	1
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

gen, according to Folin and Wu; (4) the urea nitrogen, according to Karr's direct nesslerization method; (5) and the chlorides, expressed as sodium chloride, according to the method of Whitehorn. (9) The iron and hæmoglobin were determined from whole uncoagulated blood according to the procedure of Wong, (10) and the oxygen volume calculated (7) on the basis of the hæmoglobin content. The blood serum was used in the determination of calcium and inorganic phosphate (acid soluble). The calcium was determined according to the Clark-Collip modification (1) of the method of Kramer and Tisdall, (6) and the inorganic phosphate according to the procedure of Youngburg and Youngburg. (11)

#### RESULTS AND DISCUSSION

The results obtained in this study are given in Table 1.

The data obtained are about the same as those for cattle. In so far as the same constituents are concerned, they do not differ very much from those of Posa, with the exception of the values for the serum calcium and blood sugar, where striking differences were noted. In the present work the serum calcium ranges from 9.5 to 10.5 mg per cent, with an average of 10.05 mg, and the blood sugar has an average of 44.00 mg per cent, while Posa gives a range of 26.57 to 33.44 mg per cent, with an average of 28.19 mg for serum calcium, and 73.65 mg per cent for blood sugar. The low blood sugar values in the present work may be due chiefly to the procedure(3) followed in preparing the filtrates, which leaves minimum amounts of reducing nonsugars.

#### SUMMARY

The results of quantitative chemical determinations of certain blood constituents of twenty normal Philippine carabaos are presented. These constituents, with their corresponding values as obtained in the present study, are shown in Table 2.

TABLE 2.—Summary of blood constituents of carabaos.

Constituents.	Minimum.	Maximum.	Average
	Per cent.	Per cent.	Per cent.
Blood sugar mg		55.25	44.00
Total nonprotein nitrogen mg	18.14	28.78	21.28
Urea nitrogen mg	12.44	21.80	15.19
Serum calcium mg		10.50	10.05
Inorganic phosphate mg		8.00	5.78
Chlorides as NaCl mg.	1	495.00	478.25
Iron mg.	40.16	51.81	46.09
Hæmoglobing.		15.47	18.76
Oxygen volume_	1	20.72	18.44

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### HISTOPATHOLOGY OF EARLY LESIONS IN FOURTEEN CHILDREN OF LEPERS, I

ANALYSIS OF PREVIOUS SKIN BLEMISHES IN RELATION TO SITES OF BIOPSIES AND OTHER POSITIVE AND PROBABLE LESIONS

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#### THREE PLATES

Gomez, Avellana Basa, and Nicolas, (6) in their study on 308 Culion children, concluded that

The skin is the most frequent recognizable site of early lesions of leprosy, and infection by this route is greatly favored, presumably because of the great prevalence of skin diseases among children which offer anatomical conditions favorable to the invasion of the lepra bacillus.

Gomez,(5) in a subsequent paper based on a study of the clinical histories of 100 adult lepers confined in the San Lazaro Hospital, Manila, and supplementing the work done on Culion children, amplified his previous conclusion and stated that an initial primary lesion in leprosy does not necessarily exist.

Rodriguez, (22) continuing the previous studies of Gomez et al. on Culion children, found a single cutaneous lesion, which he believed to be the initial lesion, in 75 per cent of the 59 children who had become definitely leprous, and observed 3 cases in which the enlarging macules started from scabies scars.

Neff (18) previously reported the case of a 14-month-old child with a leprotic papulondule which cleared up after seven months of treatment, presumably of intramuscular injections with moogrol.

Chiyuto, (1) in order to confirm a new orientation on leprosy studies as advocated by Manalang, (13, 14, 15) examined 45 biopsies from 40 children of lepers varying in age from  $2\frac{1}{2}$  to 20 years. He found perivascular round-cell infiltrations in 30 of them, and tuberculoid leprosy in 15. Of the 30 perivascular round-cell infiltrations, 25 were from hazy depigmented areas, 2 from depigmented macules, 2 from normal skin, and one from an anæsthetic area. Of the 15 showing tuberculoid changes, 7

were from pinkish macules, 3 from "gooseflesh" areas, 3 from pale pinkish areas with circinate borders, 1 from a coppery lesion, and 1 from a hazy depigmented area. In none of them were acid-fast bacilli demonstrated in smears from the biopsy, aside from suspicious acid-fast granules in 1 case, a pinkish macule. Apparently no search was made for the acid-fast bacillus in the tissues. In another paper(2) he mentioned having noted small, pinkish, irregular areas of papulelike formations (rather erythematous), with fine and shiny surface in 5 cases.

Lara and de Vera (9, 10) recently reported cases of very early leprosy in children, and pointed out the occurrence of an unrecognized characteristic leprotic papule which is usually positive for *Mycobacterium lepræ*, occurring very early in infants of leprous parents.

Chiyuto in a third paper (3) noted similar lesions and suggested the name "intradermal nodule" for them. No smears were taken from the lesions of his cases, and in five that were biopsied it was stated that "the tissue reaction was similar to the nodule produced by positive leprolin reaction without pus formation." From this description the lesions were apparently tuberculoid. The five biopsies were said to be "negative for M. lepræ in tissue."

#### PRESENT STUDY

The present report is based on 14 biopsies of very early skin lesions of leprosy in 14 children of lepers, varying in age from 194 months to 3 years and 4 months. The period of contact of these children with their leprous parents varied from 5 months and 12 days to 18 months. The biopsies were obtained from the 35 cases reported by one of us (C.B.L.) as having definite, or probable leprotic lesions of the skin, (8) determined by regular objective observations of the children at intervals of an average of two months or less from birth. Occasional bacteriological examinations for M. lepræ of the suspected leprous lesions by the "scraped-incision" method(28) were also made freely for confirmation. In the hacteriological examinations non-acid-fast bacilli, diphtheroids, and non-acid-fast coccoids and granules have apparently been observed and reported as either occurring alone or in association with acid-fast forms. During the regular observations all skin blemishes, like disseminated, discrete, or

<sup>&</sup>lt;sup>2</sup> Most of the bacteriological examinations were made by Dr. J. C. Manalang, of the Pathological Section.

closely set vesiculopapules with varying admixture of vesiculopustules, scabies, miliaria, mild ringworm, eczema, furuncles, wheals from insect bites, molluscum contagiosum, and so forth, were recorded, including a general regional or specific mention of their locations on the body surface. In this paper, besides the histopathological features observed in the biopsied material, an attempt was also made to analyze the individual protocols of the cases regarding the location of the previous skin blemishes in relation to the localization of the definite or probable leprotic biopsied lesion and other probable lesions, with the hope of gaining an insight into the probable genesis of these precocious lesions in very young children. These observations on previous skin blemishes are included in condensed form (under protocol) following the histological findings of each case.

The biopsies were all performed by Dr. J. G. Samson, in charge of the surgical work in Culion Colony. Only about half of the original lesion, if small, or a smaller portion of it, if larger, including some of the uninvolved skin was obtained, leaving a portion of the lesion for further clinical observations. In only one case (case 4) was the biopsy obtained from the center of the lesion, which was a hazy pale area 28 by 30 mm, with minute papules chiefly along the borders.

#### HISTOLOGICAL METHODS

The biopsied specimens were fixed in Zenker's fluid, and six micrasections made by the paraffin method. The sections were stained with eosin and hæmatoxylin, Voerhoff's stain for elastic tissues, Mallory's aniline-blue connective-tissue stain, and for acid-fast bacilli by means of a modification of Wade's method, (29) 5 per cent nitric acid being used instead of 15 per cent in the decolorization, and 1 to 3 dilutions of Loeffler's methyline blue instead of the concentrated solution, as counterstain.

In the beginning some specimens were also stained with Mallory's eosin and methylene blue, with the object of determining the possible presence of non-acid-fast bacilli in the sections, occasionally reported from the smears; but this procedure was later given up, since no apparent difference was found between this stain and the acid-fast stain in demonstrating the non-acid-fast structures. Mallory's aniline-blue stain for connective tissue was used primarily for the identification and examination of the nerves in the tuberculoid lesions as suggested by Wade. (26) Voerhoff's stain for elastic tissue was used for the identification

of the possible presence of scars in the specimens by the absence or scarcity of elastic elements in healed scars. Because of the very minute size of the lesions the entire specimens of all the cases were examined by sectioning serially the entire paraffin block and mounting the sections serially on numbered slides averaging from three to seven sections to a slide. Every 1st, 10th, 20th, 30th, and 40th or 50th slide of the series, as the case may be, was stained with eosin and hæmatoxylin; every 2d, 11th, 21st, 31st, and 41st or 51st slide was stained for elastic tissue; and every 3d, 12th, 22d, 32d, and 42d or 52d slide for connective tissue. By using these stained sections as a guide in locating the level of the serial sections where the amount of involvement of the corium by the leprous infiltration was most prominent, the slides to be stained for acid-fasts were then selected.

As control for the elastic tissue stain, a scar from the hand of a necropsied child (A.-1657), 8 months old, was used.

Search for non-acid-fast bacilli, non-acid-fast diphtheroids, coccoids, and granules was also made in the tissues of the first eight cases where they had been found. To control this part of the study biopsied skin from an adult nonleper previously infiltrated for 7\frac{1}{2} months with iodized ethyl esters of Hydnocarpus wightiana oil. (20) and normal skin from the same nonleper. were used. The locally treated control nonleprotic skin sections showed lesions histologically simulating leprosy, due to the accumulation of large numbers of vacuolated monocytes attracted by the injected oil. In this control section non-acid-fast bacilli and beaded bacillary forms, and non-acid-fast granules and coccoids were also found. None of these were found in the normal skin control. As this control was considered not adequate, the skin having been injected with oil, another skin control section on file in our laboratory, a tertiary tubercular syphilide lesion, also showing a tuberculoid histology with very few spirochætes in the sections, was studied. In this section a few non-acid-fast, faint-staining bacillary forms and a few disintegrating nuclei whose chromatin contents showed clumps of faint-staining fragmented bacillary forms were also found. Because of these findings the search for non-acid-fast bacilli and non-acid-fast coccoids in the tissues had to be given up, as it was not possible to determine their exact nature. Since this point is not the main object of this paper it should be subjected to further investigation. As far as the present observations go, these non-acid-fast, faint-staining bacillary forms and coccoids are interpreted as probably degenerated chromatin material of the numerous cells crowded in the lesions.

The search for acid-fast bacilli was most time consuming, as these bacilli were very scanty in the tuberculoid lesions. When no bacilli were found on the first attempt, several other preparations were examined. In all instances where acid-fast bacilli were found Doctor Wade, or Dr. J. Manalang, was consulted and requested to check the positive findings.

#### REPORT OF CASES

Biopsied lesions and histological findings; abstract of protocols of previous skin blemishes in relation to sites of biopsy and other probable leprotic lesions.

CASE 1; Jos. Lun., 2 years and 4 months. Thigh, left external, lower. First noted March 20, 1938, as a 1-cm or smaller, roughly rounded, palepink, rough, thickened area. Eight days later, on the day of biopsy, it was a deep-pink, thickened, hypopigmented, slightly granular area, 7 mm in diameter. Incised for smear taking only on day of biopsy; negative.

Histological,-Sections show a thin continuous layer of tuberculoid and epithelioid lesions superficially located, close to the epidermis, in contact at one end with the germinal epithelium where the germinal cells appear frayed at their bases. A few monocytes invade the germinal epithelium where it is in contact with the lesion. Lesions are richly supplied with capillaries. Intermediate and deeper layers of the corium show small and medium-sized foci and long strands of tuberculoids with a few Langhans's giant cells. Some of the smaller lesions appear isolated, the larger ones around small blood vessels. The long strands of tuberculoid lesions are in close relation and adjacent to one side only of two hairshafts, the lesions extending to the subcutaneous fatty tissue surrounding groups of sweat glands. Smooth hair muscles of two hairshafts are split and invaded by the tuberculoid lesion. The two hairshafts appear normal. Small perineural foci in the reticular layer close to the subcutis are also present, but the small nerve trunks are apparently not invaded aside from encroachment on their outer sheaths. Serial sections, however (slides 7 and 8), show in one nerve trunk actual invasion by two monocytes. This condition is found only at one level and not uniform in the course of the nerve. Round-cell collections are not a prominent feature, except slightly in the tuberculoid areas. Elastic fibers are present close to the basement membrane, in spite of the superficially located lesions very close to it and touching the epidermis at one point. No scar was found. One acid-fast bacillus was found in the lesion very near the surface (slide 7, section 1).

Protocol.—Born November 14, 1935. Separated from parents at 16 months.

Found positive (+) for *M. lepræ* from a papule in front left angle of mandible, June 29, 1938, three months after the biopsy of the left thigh lesion.

Previous skin blemishes and relation to probable lesions:

- (C) December 18, 1935. A few pin-head to pea-sized reddish vesico-papules over left of chest, left shoulder, right scapula, both sides of neck, and anterior of left thigh.
- (C) February 15, 1936. Vesicopapular eruptions on cheeks, forehead, externally on upper abdomen, and feet. Fading scars on back.
- (C) April 4, 1936. A number of drying scabieslike lesions and scars at dorsum of both feet, ankles, and legs. Numerous pale and brownish scars on trunk, arms, and forearms.
- (C) September 23, 1936. Isolated papulovesicular eruptions, back of neck, left of chest, and left knee.
- (C) February 2, 1937. Fairly numerous small, pale scars on trunk and upper extremities. Dry scabies lesion below right knee.

Separated from parents March 15, 1937, at 16 months.

- (N) March 28, 1938; probable lesions:
- (a) Posteriorly on middle of right thigh, elevated, slightly hypopigmented area, 5 mm in diameter.
- (b) Anterolaterally on upper right thigh, minute, hypopigmented, slightly raised area, about 1 mm in diameter.
  - (c) Similar lesion, posteriorly on right leg, 1.5 mm in diameter.
- (d) Externally on left thigh, one on upper portion and one on lower portion, are two deep pink, thickened, hypopigmented, slightly granular areas, the upper 6 mm and the lower 7 mm in diameter, the latter biopsied on this date.
- (e) On left lower lumbar a pink, slightly elevated, hypopigmented area, about 4 mm.

June 29, 1938.—Found positive (+)4 for acid-fast facilli, from a papule 2.5 mm in front of left angle mandible, noticed 6 days before and 3 months after biopsy of left thigh lesion. The papule was flat, brownish red, firm, slightly shiny, apparently slightly inflamed at the margin but without distinct areola.

Comment.—Previous skin blemishes were pin-head to pea-sized vesicopapules, anteriorly on left thigh in December, 1935; vesicopapular eruptions on cheeks in February, 1936, scabieslike lesions and scars on legs in April, 1936, and numerous scars on trunk since April, 1936. No previous blemishes were noted in the right thigh.

CASE 2. Moi. Miñ., 2 years and 6½ months. Posteriorly on upper right thigh. First noted as a hazy pale area about 1 cm in diameter, December 3, 1937; became 4.5 by 5 cm, but not very distinct, February 4, 1938. Two weeks after the latter date it measured 7 by 3 mm. March 8 it became a

- <sup>9</sup>(C) indicates observations made in the Colony while the children were with their leprous parents.
- \*(N) indicates observations in the Nursery, after separation of the children from their parents.

'The following scheme has been followed in grading the smears for M. lepræ: ++++, globi in practically every field; +++, one to five globi in the whole smear, and bacilli in every field; ++, no globi but bacilli quite easily found, 6 to 20 in a field; ++, no globi but bacilli found with difficulty, 1 to 5 in a field.

minute depigmented area, slightly shiny, not perceptibly elevated, 5 by 3 mm, and March 28 it was a hypopigmented, pink, slightly raised area about 5 by 4 mm, when it was biopsied. Incised for smear taking March 8 and March 28; negative for acid-fast bacilli in both instances.

Histological.—Sections show in the papillary layer six or seven small collections of large monocytes, almost side by side, showing very early epithelioid changes (Plate 1, fig. 1). They are superficially located close to the epidermis, a considerable portion of it in close contact with the germinal epithelium. From one of these collections a strand of round cells mixed with some monocytes with pink-staining cytoplasm and also showing in some cells beginning epithelioid changes (in serial sections) ensheathing a medium-sized blood vessel deeper in the section in close relation to a group of sweat glands and a minute nerve trunk (slides 8, 9, 10). The nerve trunk shows no apparent invasion of its sheath. Slight perivascular, round-cell collections are found on either side of the main lesion. A scar in the deeper serial sections (probably the incision of March 8 for smear taking) extends from deep down the corium to the surface. In the intermediate zone of the corium there is one minute collection of large monocytes and small round cells richly supplied with four capillaries. One or two cells in the collection show beginning epithelioid changes, while some are apparently undergoing mitosis. This small lesion is in relation to a small blood vessel and a duct of a sweat gland. No definite tuberculoid lesion was found. Hairshafts appear normal and uninvolved. The subcutis is also uninvolved. One giant cell is found in the depth of the scar, none in the monocyte collections. Two doubtful, short acid-fast rods (?) in a cell in the lesion (slide 16, section 3).

Protocol.—Born September 4, 1935. Separated from parents at 162 months. Never found positive for M. lepræ in smears up to the day of biopsy.

Previous skin, blemishes and relation to probable lesions:

- (C) December 17, 1935. Fairly numerous reddish and brownish pin-point to pin-head vesicopapules on abdomen, flanks, and chest.
- (C) February 15, 1936. Ulcerated, large eruptions, probably scabies, on feet, hands, and back of head; a few scattered on trunk and other portions of extremities.
- (C) April 3, 1936. Fairly numerous dark-brown scars on feet and lower legs.
- (C) May 26, 1936. A few corn-sized superficial ulcers, presumably scabies, on dorsum of both feet. A few brown scars on both feet, around ankles, and on abdomen.
- (C) September 22, 1936. A few reddish papular eruptions on feet, especially around ankles.

Separated from parents January 21, 1937, at 162 months.

- (N) October 1, 1937. Minute pale scars medially on right buttock and posteriorly on upper right thigh.
- (N) December 3, 1937. Minute depigmented area, medially on right buttock and posteriorly on right thigh. Hazy pale area about 1 cm in diameter, posteriorly on upper right thigh.
- (N) February 4, 1938. Small depigmented area just below right buttock, 3.2 mm in diameter, similar small area medially on right buttock.

Previous hazy pale area posteriorly on upper right thigh not very distinct, but apparently larger, 45 by 50 mm. On anteromedial upper right thigh a pale pinkish area, very slightly elevated, 3 by 4 mm.

(N) March 28, 1938; probable lesions:

- (a) Hypopigmented, pink, slightly raised area, posteriorly on upper right thigh, 5 by 4 mm (biopsied).
  - (b) Similar lesion on middle of left buttock, about 2 mm.
  - (c) Minute depigmented spot, medially on right buttock, about 2.5 mm.
- (d) Pink, indurated, distinctly elevated area, 6.5 by 3.5 mm, anteromedially on right thigh.

Comment.—Previous blemishes were a few ulcerated, large eruptions, probably scabies, scattered on extremities, in February, 1936; minute pale scars medially on right buttock and posteriorly on upper right thigh in October, 1937, which apparently became depigmented and hazy pale areas in December, 1937, and February, 1938; by March 28, 1938, these skin blemishes appeared as described above under that date (lesions a and c). No previous blemishes were noted in the left buttock.

CASE 3. BUE. MIA, 1 year and 8½ months. Leg, left, postmiddle. First noted March 7, 1938, as an indurated, thickened, brown, scarlike spot within an area of closely grouped scars. March 28, when it was biopsied, it was a dark, indurated scar, 11 mm in diameter. Incised for smear taking March 7 and March 28, 1938, negative for acid-fast bacilli in both instances.

Histological.—The section shows a small, definite scar from the depth of the corium to the epidermis, near one end of the section only, probably from smear taking March 7, 1938. The very superficial thin strip of corium beneath the epidermis through the length of the section, except a small portion at one end, shows evidence of superficial scarring by the complete absence of elastic fibers and the presence of many small blood vessels and numerous, fairly young, connective tissue cells. The epidermis shows uniform parakeratosis throughout. Immediately beneath this long and very thin strip of superficial scar, the papillary and reticular layers of almost the entire section, except at one end, appear cellular, due to a more or less extensive diffused distribution of round cells and large monocyte collections around blood vessels, two hairshafts, and sweat-gland ducts. No definite epithelioid or tuberculoid changes. Very small nerve trunks in the intermediate zone show no apparent changes. Serial sections show the same condition, except that the deepest coils of sweat glands near the subcutis are also involved. In one section close to a hairshaft very early evidence of apparent epithelioid changes is noted, and deeper down in the same section are three large collections of round cells and large monocytes in relation to coils of sweat glands. No definite tuberculoid lesion was found. Hairshafts show no changes. No giant cells. Probably a very young lesion about to become a tuberculoid. Two acid-fast bacilli found in a cell in the lesion (slide 3, section 1).

Protocol.—Born July 14, 1936. Separated from parents January 21, 1938, at 18 months. Up to the day of biopsy not found positive for M. lepræ in smears.

Previous skin blemishes and relation to probable lesions:

(C) August 5, 1937. Small, pea-sized, eroded vesico-papules, externally on thighs.

- (C) October 2, 1937. Fresh scars with brownish background, rather extensive anteromedially and posteriorly on left leg and to a less extent anteriorly on right leg. A few pea-sized to bean-sized shallow ulcers at the same region. Fresh scabieslike lesions below right knee and externally on left cheek.
- (C) December 4, 1937. Very extensive area of scar involving practically the whole left leg with a number of pea- to bean-sized superficial ulcers still present posteriorly and medially on left leg.

Separated from parents January 21, 1938, at 18 months.

(N) March 7, 1938. An indurated, thickened, brown, scarlike spot within an area of closely grouped scars posteriorly on middle of left leg.

(N) March 28, 1938; probable lesion:

Only one, posteromedially on left leg a dark, thickened, indurated scar, 11 mm in diameter (biopsied). No other suspicious lesions.

Comment.—Previous skin blemishes were extensive scars posteriorly on left leg and practically the whole left leg in October, 1937, and December, 1937; a thickened, indurated, brown, scarlike spot posteriorly on left leg, March 7, 1938, biopsied March 28, 1938. This lesion was definitely located beneath a scar, identified also histologically.

CASE 4. SUL. DIA., 1 year and 11 months at time of biopsy. Posterolaterally on upper right thigh. First noted February 4, 1938, as a pale area about 25 by 15 mm, slightly granular in the lower portion, suggesting follicular hypertrophy. Two weeks later the area had increased to 25 by 25 mm, with several pin-point to pin-head micropapules at the postero-inferior border, which were slightly reddish. March 28, 1938, this lesion measured 28 by 30 mm, still with minute papules chiefly along the borders. Biopsy March 29, taken from the center of the lesion. Incised for smear taking February 4, 1938, and March 28, 1938; negative for acid-fast bacilli in both instances.

Histological.—A very small scar near center of section, close to fresh incision of March 28, showing many dilated empty blood vessels. Scar relatively fibrous and cellular, embedding atrophic and regenerating hairshafts probably injured in the first incision, February 4, 1938. Perivascular round-cell infiltration present, slight to moderate in superficial papillarv laver of entire section. Smooth hair muscles with marked round-cell infiltration, splitting the muscle fibers (Plate 1, fig. 2); infiltrating cells mainly small, round cells and very few large monocytes. Deeper reticular layer clean, without lesions, except for some polynuclears infiltrating, apparently due to incision for smear taking, the day previous to biopsy. Intermediate zone of corium at one end showing slight perivascular round cells in relation to a smooth hair muscle, small nerve trunks, and a group of sweat-gland coils. Small nerve trunks and sweat glands in deeper corium uninvolved. No evidence of tuberculoid, or early epithelioid changes. Taken from center of hazy pale area with minute papules along borders. One acid-fast bacillus found in a round-cell collection (slide 11, section 1).

Protocol.—Born April 20, 1936. Separated from leprous parents at 13 months. Up to the day of biopsy not found positive for M. lepræ in smears. Previous skin blemishes and relation to probable lesions:

(C) September 23, 1936. Moderate amount of papulovesicular eruption on arms, elbows, buttocks, and a few eruptions on hands and feet.

- (C) December 4, 1936. A number of minute, closely-set, brown scars on trunk and extremities.
- (C) February 2, 1937. Numerous minute, closely-set, brown scars on trunk and extremities; minute active scabies on both feet and ankles.

April 2, 1937. Dark-brown scars of scabies, both feet and lower legs. Thickened, scaling, fresh scars of scabies, anteriorly on left leg and above left heel. Pea-sized, eroded papulopustules, left posterior axillary fold, left elbow, and left buttock.

Separated from parents, May 15, 1937, at 13 months.

- (N) August 4, 1937. A number of pin-head or smaller vesicopapules on hands, anteriorly on thighs and feet, and posteriorly on right thigh.
- (N) December 3, 1937. A number of small dark-brown scars, scattered on trunk and extremities; no active skin eruptions.
- (N) February 4, 1938. Posterolaterally on right thigh a pale area, about 25 by 15 mm, slightly granular on lower portion, suggesting follicular hypertrophy.
  - (N) March 26, 1938; probable lesions:
- (a) Posterolaterally on right thigh a hazy pale area, about 28 by 30 mm in diameter, with minute papules chiefly along borders (biopsied).
- (b) Posteriorly on upper left thigh a minute brownish-red papule, 3 mm in diameter, rather firm, apparently surrounded by a faint brownish halo. Comment.—Previous skin blemishes were closely set scars on trunk and extremities, in December, 1936, and February, 1937; pin-head vesicopapules anteriorly on thighs and posteriorly on right thigh, August 4, 1937, and December 3, 1937; dark-brown scars scattered on trunk and extremities.

CASE 5. Rod. ABE., 1 year and 11½ months. Above left elbow. First noted February 26, 1938, as a pale pinkish, shiny, distinctly thickened area, 5 by 4 mm. March 31, 1938, this became distinctly hypopigmented, shiny, slightly pinkish, definitely thickened, 6 by 5 mm. Biopsied on this day. Incised only on day of biopsy for smear taking and found negative.

Histological.—Papillary layer of corium over a long stretch, showing 8 to 9 small epithelioid and tuberculoid foci side by side, in close contact with epidermis in places where many of the germinal cells show fraying of the basal cytoplasm and infiltration with occasional monocytes. (Plate 1, fig. 3). Papillary and reticular layers with fairly extensive sprinkling of tuberculoid and epithelioid foci in close relation with small blood vessels, ducts, and coils of sweat glands. In serial sections some of the subpapillary lesions evidently connected with some of the small superficially located papillary foci. Both borders of lesion apparently ending quite abruptly as smaller epithelioid lesions. No conspicuous round-cell collections on either margin. Round cells only slight to moderate in the epithelioid foci. No scar, elastic fibers present beneath epidermis in spite of the very superficially located lesions close to the germinal epithelium. Two small nerve trunks in the deep reticular layer (slides 15 and 25) surrounded and their fibers invaded and infiltrated by a tuberculoid lesion. A medium-sized nerve trunk in subcutis (slides 34 and 38) showing partial to complete tuberculoid infiltration of nerve fibers at different levels (Plate 2, figs. 5 and 6). This subcutaneous nerve in serial sections is a continuation of one of the infiltrated smaller trunks in the reticular layer. Two acid-fast bacilli in the deepest lesion (slide 4, section 2).

Protocol.—Born April 21, 1936. Separated from leprous parents at 13 months. Found positive (+) for M. lepræ in right knee, February 4, 1938, almost 2 months before the biopsy of left elbow lesion.

Previous skin blemishes and relation to probable lesions:

- (C) September 23, 1936. Anterolaterally on right leg several minute papulovesicular eruptions, medially on side of right leg a large bluish erythematous area with thickened border.
- (C) December 4, 1936. A number of pale-brown scars on upper back; a few pin-head, reddish vesicopapules on shoulders, anterior axillary folds, and above knees. Minute scaling on dorsum of both feet.
- (C) February 2, 1937. Minute healing scabies on right big toe and external malleoli.
  - (C) April 2, 1937. A few small, fresh scars on feet and lower legs. Separated from parents, May 22, 1937, at 13 months.
- (N) August 4, 1937. Very hazy pale mottlings anteriorly on legs and anterolaterally on thighs.
  - (N) October 1, 1937. Anterolaterally legs practically normal.
- (N) February 4, 1938. One inch below left popliteal a reddish, wheal-like, slightly shiny, raised area, about 5 mm in diameter. On upper part of left knee a small, pale, rather shiny, thickened area, apparently with subsiding central puncture, 5 mm by 3 mm. Medially on right knee a similar lesion, about 4 mm in diameter. The latter positive (+) for M. lepræ.
- (C) February 26, 1938. Just above the left elbow a pale pinkish, shiny, distinctly thickened area, 5 by 4 mm, suspicious.
  - (C) March 31, 1938; positive and probable lesions:
- (a) Previous lesion below left popliteal, still pinkish, distinctly thickened, 6 by 4 mm.
- (b) Lesion above left elbow distinctly hypopigmented, shiny, slightly pinkish, definitely thickened, 6 by 5 mm (biopsied on this day).
- (c) Lesion over upper left knee distinctly pinkish and thickened, slightly shiny, 5 by 4 mm.
- (d) Previous positive lesion medially on right knee now a dark-brown scar, infected and scratched after smear taking, February 4, 1938.
- (e) On upper right knee a slightly thickened area, not distinctly hypopigmented, 9 by 6 mm.

Comment.—Previous blemishes at sites of probable lesions were a few pin-head, reddish vesicopapules above knees, in December, 1936, and fresh scars on lower legs, April, 1937. No previous blemishes noted on left elbow

CASE 6. ERN. VIB., 1 year and 9 months. Right thigh, upper, external. First noted February 4, 1938, as a pinkish, slightly shiny papule, about 3 mm in diameter, surrounded by a narrow pale halo. Positive (++) for M. lepræ. March 12, 1938, the lesion was still fairly distinct, 3 mm in diameter, pinkish, slightly wrinkled and surrounded by a pale halo when it was biopsied. Incised once for smear taking, February 4, 1938.

Histological.—In the papillary layer the section shows a very thin continuous layer of epithelioid and tuberculoid lesion (slides 2, 5, 7) for a length of about 1.5 mm in close contact in places with the germinal epithelium over which elastic tissue is absent for a length of about 1.2 mm.

The overlying epidermis for 2.3 mm is flattened over it. This possibly represents a portion of the incision scar of February 4, 1938. At about the middle of the papillary layer the lesion extends downward between two closely set hairshafts to the depth of the reticular layer, forming infiltration around small nerve trunks, smooth hair muscles, blood vessels. and a small group of sweat-gland coils in the deepest margin of the reticular layer. In the subpapillary and reticular layers on either side of the two hairshafts mentioned are small collections of epithelioids in relation to small blood vessels, and infiltrating smooth hair muscles. Deeper serial sections show larger epithelioid and tuberculoid lesions in the papillary and reticular layers, and a deep scar (incision February 4, 1938) near the border of the lesion. A small nerve trunk in the deeper corium shows invasion of the nerve fibers with large monocytes (slides 5, 6, 7). On either side of the lesion, where it joins the normal skin, the transition as smaller epithelioid lesions appears to be abrupt. Several acid-fast bacilli easily found in both superficial and deeper lesions (slide 4, section 1).

Protocol.—Born June 12, 1936. Separated from leprous parents at 13 months. Found positive (++) for M. lepræ from a papule externally on upper right thigh, February 4, 1938, a little over 1 month before its biopsy. March 12, 1938.

Previous skin blemishes and relation to probable lesions:

- (C) December 8, 1936. A number of pin-head reddish wheals on cheeks; fairly numerous pin-head or larger dark-brown spots posteriorly on forearms, anteriorly on legs, externally on right thigh, probably subsiding wheals. Red wheal on right hip.
- (C) February 2, 1937. On left side of chest a small, pea-sized, dark-brown, thickened scar.
- (C) June 2, 1937. A few dark-brown scars on left side of chest and left buttock; dark-brown scars posteriorly on right thigh, right side of chest, medially on right arm.

Separated from parents, June 19, 1937, at 13 months.

- (N) December 3, 1937. A number of dark-brown scars on legs, thighs, and buttocks.
- (N) February 4, 1938. Minute reddish wheal on upper right cheek below malar and erythematous spot on middle of left cheek. On upper external right thigh, about 1 inch below, and a little posterior of major trochanter, a pinkish, slightly shiny papule about 3 mm in diameter, surrounded by a pale halo, slightly suspicious [positive (++) for M. lepræ on this date]. Returned to parents in the Colony, February 7, 1938.
- (C) February 12, 1938. On middle of right cheek a pale pinkish, thickened area, 4 mm in diameter, with a very hazy pale halo.
- (C) February 26, 1938. (a) A red, flat papule on right cheek, 4 mm in diameter. (b) A papule posterolaterally on upper right thigh, 4 mm in diameter, shiny, slightly wrinkled, deep pink. (c) Anteriorly on upper right forearm, a reddish, very slight elevated area, also 4 mm in diameter, surrounded by a very faint, pale halo.
  - (C) March 12, 1938; positive and probable lesions:
- (a) On right cheek, quite thin, almost imperceptibly elevated, distinctly pinkish, 6 by 5 mm.
- (b) Positive lesion, posterolaterally on upper right thigh, still fairly distinct, 3 mm in diameter, pinkish, surrounded by a pale halo (biopsied).

(c) Anteriorly on upper right forearm a brownish spot, about 3.5 mm in diameter, almost imperceptibly elevated, apparently subsiding, indurated.

Comment.—The previous blemishes mentioned were reddish wheals on cheeks, and fairly numerous pinkish, larger, dark-brown spots, posteriorly on forearms and externally on right thigh in December, 1936; dark-brown scars posteriorly on right thigh in June, 1937, and on thighs in December, 1937.

CASE 7. EPI. GAL., 1 year and 8 months. Posteromedially on right arm. First noted February 4, 1938, as a pin-head, shiny, pinkish papule, surrounded by a very pale, reddish zone, positive (+++) for M. lepræ on this date. Apparently not present in the last previous examination, December 3, 1937 (see protocol below). February 25 this pin-head papule was 3 mm in diameter, surrounded not by a red areola but by a narrow pale halo. March 12, when it was biopsied, it was 4 mm in diameter and distinctly thickened. Incised once for smear taking, February 4, 1938.

Histological.—Sections show for a stretch of about 3 mm in the papillary layer a continuous uninterrupted layer of epithelioid lesions of moderate thickness, in close contact with the epidermis in places where the basal cytoplasm of the germinal cells is fraved and invaded by a few monocytes. Elastic fibers beneath epidermis are absent for a stretch of 1 mm in the center of the involved papillary layer (slide 2), where three moderatesized blood vessels are present side by side at this level close to the epidermis but within the epithelioid lesion. Epidermis flattened over scar, and for some distance on either side of it. This flattening possibly represents only a small portion, the border of the incision scar of February 4, 1938. In all the other serial sections examined there is no evidence of deep scarring. A strand of epithelioid lesion extends deeply into the subpapillary reticular layers, infiltrating a smooth hair muscle around a hairshaft, sweat gland duct, and deep coils of the sweat-gland duct. Deeper sections show isolated small and medium-sized epithelioid and tuberculoid lesions. Small nerve trunks show perineural epithelioid collections, and in two the nerve sheaths are involved by the perineural epithelioid collections (slide 9). The lesion having been incised for smear taking February 4, the very superficial thin scar noted may represent only the edge of the incision scar included in the biopsy. Many acid-fast bacilli found (slide

Protocol.—Born July 11, 1936. Separated from leprous parents at 16 months. Found positive (+++) for M. lepræ posteromedially on the right arm, February 4, 1938, at 1 year and 7½ months.

Previous skin blemishes and relation to probable lesions:

- (C) September 24, 1936. Plenty of minute papular eruptions on upper eyelids, cheeks, especially on trunk, anteriorly and posteriorly, a few on extremities where minute dark scars are more distinct.
- (C) December 7, 1936. No active skin eruptions, but a number of small dark-brown spots on back, either scars or traces of previous wheals.
- (C) February 3, 1937. Pea-sized, subsiding, reddish, inflamed papule posteriorly on right leg. Raised reddish thickened scar on right elbow.
- (C) April 3, 1937. A number of minute vesicopapules on both buttocks. Drying, bean-sized, crusted ulcers posteriorly on right leg.

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(C) October 2, 1937. Fairly numerous fresh, thickened, dark-brown, small scars anteriorly on legs, especially on left knee and a few on feet, externally on thighs, ulnar side of wrists, and above coccyx.

Separated from parents November 23, 1937, at 16 months.

- (N) December 3, 1937. Numerous pea- to bean-sized, slightly raised, drying, healing ulcers, probably scabitic, on feet, legs, thighs, both forearms, and on arms and buttocks. On buttocks fairly numerous dark-brown scars.
- (N) February 4, 1938. Posteromedially on right arm a pin-head, shiny, pinkish papule, surrounded by a very pale reddish zone, distinctly suspicious. Positive (+++) for M. lepræ. Returned to parents in the Colony, February 7, 1938.
- (C) February 12, 1938. Anterolaterally on right thigh about middle portion a pink shiny papule, 3 by 5 mm, surrounded by a faint, pale halo.
- (C) February 25, 1938. Red shiny papule, 3 mm in diameter, posteromedially on right arm and 4 cm above elbow. Externally on lower right thigh, 5 cm above knee, another red papule, about 5 mm. Positive (++) for acid-fast bacilli.
  - (C) March 12, 1938; positive and probable lesions:
- (a) Positive lesion, posteromedially on right arm, now a shiny pin-head papule, 4 mm in diameter, distinctly thickened (biopsied).
- (b) Positive lesion anterolaterally on right thigh, now a purplish papule about 5 mm in diameter, surrounded by a narrow pale halo.
- (c) On the lower left buttock a pinkish papule about 3 mm in diameter, slightly indurated, not surrounded by a pale halo.

Comment.—Previous blemishes were papular eruptions on extremities with minute dark scars, in September, 1936; vesicopapules on both buttocks in April, 1937; thickened dark-brown scars, externally on thighs in October, 1937; and healing ulcers, probably scabitic, on thighs, arms, and buttocks, with fairly numerous dark-brown scars on buttocks in December, 1937.

CASE 8. EDI. URO., 2 years and 8 months on date of biopsy. Anteromedially on left leg, two inches above ankle. First noted February 4, 1938, as a small papule, 3 mm in diameter, resembling a wheal, shiny, very slightly pinkish, surrounded not by a red areola but by a very faint, pale halo. February 19 it was 4 by 2 mm, surrounded by a hazy, pale halo 12 by 11 mm. March 8 it was 5 by 4 mm, pale pinkish, shiny, slightly elevated, surrounded by faintly hypopigmented skin, 10 mm in diameter. March 28 it was 5 by 6 mm, shiny, slightly elevated, very slightly hypopigmented, slightly indurated. Positive (+) for M. lepræ in smears on this date, when it was biopsied. Incised for smear taking February 4 and March 28, found positive only on latter date.

Histological.—Biopsied specimen thin, subcutaneous tissue not included. Sections show the lesions at one end traversed by a healed, deep scar, due to previous smear taking February 4, over which the epithelium is flattened. On either side of the scar in the papillary layer are epithelioid foci, side by side, fairly rich in capillaries which are congested. In deeper serial sections these superficially located lesions are in contact (slide 10) with the germinal epithelium, whose cells show fraying of their basal cytoplasm in places. Subpapillary and reticular layers on either side of scar dotted with epithelioid and tuberculoid lesions, some of lesions in subpapillary

layer appearing in serial sections as direct extensions from the more superficially located lesions. As in previous cases, the lesions are in relation to hairshafts, small blood vessels, and sweat gland ducts. One small nerve shows a perineural tuberculoid lesion encroaching on its sheath. One acid-fast bacillus found in a large deep lesion (slide 1, section 1).

Protocol.—Born July 21, 1935. Separated from parents to relatives outside the Colony about the end of October, 1935, at 3 months. Returned to Colony Hospital with parents for colitis and pneumonia February 4, 1936, and separated again from leprous parents April 16, 1936, upon recovery at the hospital, at 9 months. Actual total period of exposure, 5 months and 12 days. Found positive in smears (+) for M. lepræ, March 28, 1938, anteromedially on left leg, the day of biopsy.

Previous skin blemishes and relation to probable lesions:

(C) April 3, 1936. Occasional reddish wheals on left leg and on right side of back.

Separated from parents for second time April 16, 1936, at the age of 9 months, after hospitalization for 2 months and 12 days.

- (N) July 28, 1936. Minute healing vesicopapules on lower legs, and fresh pin-head to larger vesicopapules on lower back and both sides of chest and medially on arms and chest.
- (N) September 21, 1936. Skin rough where there are papulo-vesicular eruptions at upper trunk, arms, legs, and ankles.
- (N) December 3, 1936. Fresh scars medially on right foot. Groups of a few pin-point or pin-head vesicopapules, not congested on right flank, right infra-axillary, and left interscapular.
- (N) February 1, 1937. Subsiding, scaling, minute vesicopapules on buttocks and around ankles.
- (N) March 17, 1937. Several whitish pin-head papules at knees. Feet dry with several small vesicopustules.
- (N) June 1, 1937. Patch of eczematoid dermatitis on ulnar side of left wrist and posteriorly on lower right leg. Group of small scars anteriorly on lower left leg.
- (N) February 4, 1938. Anteromedially on left leg, 2 inches above ankle, a small papule, shiny, resembling a wheal, very slightly pinkish, 3 mm in diameter, suspicious. Smears taken on this day negative for acid-fast bacilli.
- (N) February 19, 1938. Above lesion now 4 by 2 mm, surrounded by a hazy, pale halo, 12 by 11 mm.
- (N) March 8, 1938. Above lesion, pale pinkish, shiny, slightly elevated. now 5 by 4 mm.
  - (N) March 28, 1938; probable lesion:

Only one, above lesion, anteromedially on left leg, shiny, slightly elevated, slightly indurated, very slightly hypopigmented, now 5 by 6 mm (biopsied). No other lesion. Positive (+) for only one acid-fast bacillus.

Comment.—Previous skin blemishes on left leg reddish wheals in April, 1936, healing vesicopapules in July, 1936; papulovesicular eruptions in September, 1936; and a group of small scars in June, 1937. Total period of exposure of this child was 5 months and 12 days.

CASE 9. ANA. MAN., 1 year and 7½ months on date of biopsy. Above right knee. First noted February 4, 1938, as a pale pinkish area, about

7 by 5 mm, very slightly raised. March 8, 1938, it was still fairly distinct as a faint, light-purplish, elevated area, 10 by 5 mm, with the center more deeply purplish and showing a linear scar due to incision for smear taking February 4, the whole area very slightly hypopigmented. March 28, the day of biopsy, it was 12 by 5 mm, hypopigmented, slightly pinkish, slightly elevated, with depressed, deep-pink, shiny center, site of previous smear taking. Incisions for smears February 4, March 9, and March 28, 1938, all negative for acid-fast bacilli. The remaining lesion left in the child for further clinical observation became positive (+) June 4, 1938, a little over 2 months after biopsy.

Histological.—Sections show a small scar (incisions of February 4 and March 9) in the center of the lesion, extending from about the middle of the corium to the epidermis. Below the scar and on either side of it, around blood vessels and hairshafts, are small and medium-sized epithelioid and tuberculoid lesions, with infiltration of the smooth hair muscles, sweat glands and ducts, and small nerve trunks. In the papillary layer are three or more small epithelloid foci and slight pervicascular infiltrations, one of the epithelioid foci in close contact with germinal epithelium, producing fraying of basal cytoplasm of their cells. This point is close to the incision scar mentioned above (slide 7). Deeper sections (slide 15) show two other, very small epithelioid foci similarly in contact with the germinal epithelium. Some of the small nerve trunks in the lesions, although appearing as still intact except for their obscure epineural sheath, show in the serial sections definite epithelioid invasion in spots of their epineural sheath, where one or two monocytes (Plate 2, fig. 7) may be seen in the nerve trunks within the capsule (slides 6, 7, and 8). In the aniline-blue sections a small nerve in the deep corium shows definite invasion of the nerve trunk when traced in serial sections (slides 6, 7, and 8, serial), and the delicate wavy-blue-staining connective tissue appears dispersed and frayed by the cellular infiltration. Several acid-fast bacilli found in superficial and deep lesions (slide 4, section 1).

Protocol.—Born August 14, 1936. Separated from parents at 17 months. Previous skin blemishes and relation to probable lesions:

- (C) June 2, 1937. A number of minute healing vesicopapules on dorsum of feet and on left ankle.
  - (C) August 6, 1937. A few minute brown scars on feet and lower legs.
- (C) October 2, 1937. A few minute pale scars on buttocks and brown scars on left arm, forearm, lower legs, and feet. On left cheek a group of minute dark-brown scars, site of previous eruptions.

Separated from parents January 19, 1938, at 17 months.

- (N) February 4, 1938. Pea-sized, fresh, pinkish scars on dorsum of right forearm, and similar dark-brown scars on feet, anteriorly on ankles, posteriorly on legs, and buttocks. Bean-sized, dry ulcer posteriorly on left thigh. One inch above right knee a pale pinkish area, about 7 by 5 mm, very slightly raised, suspicious.
- (N) February 19, 1938. Above lesion of February 4, pinkish, slightly shiny, elevated, 8 by 3.5 mm, showing a very narrow, faint, pale halo.
- (N) March 8, 1938. Above lesion of February 4, a fairly distinct, faint, light purplish, elevated area, 10 by 5 mm, very slightly hypopigmented, with a linear scar in center from smear taking.

- (N) March 28, 1938; probable lesions:
- (a) Above lesion, above right knee, now 12 by 5 mm, hypopigmented, slightly pinkish, slightly elevated (biopsied).
- (b) On ulnar aspect of middle right forearm a hypopigmented, pinkish, very slightly thickened, slightly indurated area, 10 by 12 mm, a new suspicious lesion.

Comment.—No previous blemishes noted above right knee, the site of biopsy. Previous blemishes on dorsum of right forearm were pea-sized, fresh, pinkish scars, in February, 1938.

Case 10. Ren. Tab., 2 years and 3 months old on day of biopsy. Posteriorly on right leg. First noted February 12, 1938, as a purplish, scarlike area, apparently markedly indurated, positive (++) for M. leprx on this date. February 26 this lesion resembled a dark-brown, shiny scar, slightly raised and distinctly indurated, the whole area 10 mm in diameter. March 12, 1938, when it was biopsied, this lesion was a purplish-looking scar, thickened, elevated, about 9 mm in diameter, markedly indurated deeper in the skin. Incised once for smears, February 12, 1938.

Histological.—Section shows a very superficially located and very thin scar involving a thin stretch of the papillary layer, 2 mm long (slide 3), with many dilated blood vessels (Plate 3, figs. 8 and 9), some with thin walls, others thick-walled. In all serial sections examined this superficial thin scar was present, and in no instance was it found to involve the reticular layer. In the papillary and subpapillary layers are closely set epithelioid and tuberculoid lesions extending deeply into the lower-most portion of the reticular layer and for some distance on either side of the superficial scar (Plate 3, figs. 8 and 9). The epithelioid and tuberculoid lesions are so extensive and closely set that one smooth hair muscle is found split extensively, groups of its fibers being separated for some distance from each other and invaded by the proliferating epithelioid cells. The superficially located epithelioid lesions in the papillary layer below the superficial scar appear intact and undisturbed and in close contact with the germinal epithelium at one point at one end of the very superficial scar (slide 6). A few Langhans's giant cells and prominent round-cell infiltrations in the large tuberculoid lesions in the deep corium. Perivascular round-cell infiltration slight, and not a prominent feature at the edge of the lesion where it joins the normal skin at one end. In the aniline-blue sections small nerve trunks in the smaller perivascular epithelioid collections near the border of the lesion (slides 4 and 12), but none in the larger, closely set lesions. These nerve trunks show early involvement of their epineural sheaths. Many acid-fast bacilli of variable morphology, some long (Plate 3, fig. 10), some short, some granular, and others segmented (Plate 3, fig. 11) (slide 1, section 3).

Comment.—Whether the superficial thin scar here noted was the original scar observed clinically before the incision was made for smear taking, February 12, or the result of the incision made on that date, cannot be definitely stated. As the epithelioid and tuberculoid lesions immediately beneath this superficial scar appear undisturbed and no scar deep enough can be found in all the serial sections examined, it is probable that this superficial thin scar was the original scar before the incision was made, and that the incision scar was not included in the biopsy.

*Protocol.*—Born December 14, 1935. Separated from parents at 15 months. Positive (++) in the upper right thigh and (+) in the lower right thigh February 4, 1938, and (++) posteriorly on right leg, February 12, 1938, the last the site of biopsy, March 12, 1938.

Previous skin blemishes and relation to probable lesions:

- (C) April 3, 1936. Scattered superficial ulcers, probably of scabies, on feet, right leg, forearms, hands, and right side of nose. Crusted eruptions, superiorly on scalp.
- (C) May 22, 1936. Just recovering from extensive scabies of trunk and extremities. No further active lesions of scabies.
- (C) July 29, 1936. Numerous minute pale scars on trunk and extremities, due to extensive scabies. Drying superficial ulcers on right forearm, posteriorly on right thigh, apparently also scabies.
- (C) September 22, 1936. Ulcerated scabies eruptions on legs and feet, occasional at left elbow and right axillary fold. Many small pale scars on thighs, abdomen, upper trunk, and arms. No suspicious areas.
- (C) December 4, 1936. A number of drying scabies on left knee, anteriorly on right leg, on dorsum of right foot, above left external malleolus. Numerous pale scars over trunk and extremities.
- (C) February 9, 1937. Fairly numerous pale scars on trunk and extremities.

Separated from parents March 20, 1937, at 15 months.

- (N) April 1, 1937. Numerous small pale scars on trunk and extremities. Pea-sized, eroded, slightly inflamed papules one on each lower buttock.
- (N) June 1, 1937. Fairly numerous old scars on trunk and extremities. A number of drying vesicopapules on feet and lower legs.
- (N) October 11, 1937. A number of drying superficial ulcers on dorsum of left foot and anteriorly on left leg, probably scabies. Numerous closely set, pale scars anteromedially on legs, lower forearms, upper arms, and shoulders. Fewer similar scars on thighs and trunk. Pin-head reddish vesicopapules externally on lower left buttock.
- (N) February 4, 1938. Posteromedially on upper right thigh a pinkish-pale, slightly elevated area not surrounded by an inflammatory zone, rather purplish at the middle, 8 by 4 mm, slightly suspicious, positive (++) for acid-fast bacilli. Anteromedially on the lower right thigh, 1 inch above knee, a similar, less distinct area, more definitely suggestive of a subsiding scar but still not distinctly like a scar; this area 5 mm in diameter, with deep purplish, wrinkled center, positive (+) for acid-fast bacilli. Posterolaterally on left arm a reddish-pale papule, about 7 mm, surrounded by a faint pale halo.

Returned to parents in the Colony February 7, 1938.

- (C) February 12, 1938. Posteriorly on right leg, just above middle, a pale scar, at the lower border of which is a pin-head, brownish papule. One inch below is a purplish scarlike area which seems markedly indurated, positive (++) for acid-fast bacilli.
- (C) February 26. New lesions: medially on left knee a pin-head, reddish, flat papule. Medially on upper left knee a similar, slightly larger. reddish papule with slightly wrinkled surface. Externally on upper left thigh a pinkish, shiny, slightly elevated area, 4 mm in diameter. The pre-

vious lesion posteriorly on right leg now resembling a dark-brown, shiny scar, but slightly raised and distinctly indurated, 10 mm in diameter.

March 12, 1938; positive and probable lesions:

- (a) On left arm, inferiorly and posterolaterally, a pinkish, shiny lesion, 9 mm in diameter.
- (b) On right thigh, superiorly and posteromedially, elevated, 8 mm in diameter, positive (++) February 4, 1938.
- (c) On right thigh, anteromedially, distinctly thickened, 7 mm in diameter, positive (+) February 4, 1938.
- (d) On left knee, superiorly and medially, shiny, light pinkish, elevated, 6.5 by 5 mm.
  - (e) On left knee, medially, pink, slightly elevated, 4 mm in diameter.
  - (f) On left thigh, superiorly and externally, pink, 6 mm in diameter.
- (g) On right leg, posteriorly, over purplish-looking scar, about 9 mm in diameter, positive (++) February 12, 1938 (biopsied).

Comment.—Two lesions, one anteromedially on lower right thigh (noted February 4, 1938) and one posteriorly on right leg (noted February 12, 1938), both positive for M. lepræ apparently beneath scars or scarlike areas. In the latter, which was biopsied, the original scar was confirmed histologically (Plate 3, figs. 8 and 9). Previous extensive scabies and scars recorded in all regions, showing positive and probable lesions.

CASE 11. VIR. REN., 2 years and 2 months old on day of biopsy. Externally on left lower thigh, 1.5 inches above knee. First noted December 3, 1937, as a small, pale pinkish, very slightly elevated area, about 6 mm in diameter. February 4, 1938, this was still a pale, shiny area, about 8 mm by 6 mm, with scar of incision for smear taking made December 3, 1937. March 28, 1938, it was a pinkish, slightly elevated, rough, slightly indurated area, also hypopigmented, 8 mm in diameter, with pin-point, purplish areas on its surface. Biopsied the next day. Incised December 3, 1937, and on day previous to biopsy, March 28, 1938; negative in both instances.

Histological.—Section shows a small scar, 1.4 mm in diameter (incision of December 3, 1937), extending from middle of corium to surface. Below and to one side of this scar are small foci and strands of epithelioid and tuberculoid lesions in relation to small blood vessels, hairshafts, sweatgland ducts, and sweat-gland coils. Papillary zone shows several small, perivascular, epithelioid foci (slide 15), some of which are in close contact with the germinal epithelium, causing fraying of the basal cytoplasm in some of the cells. One isolated small nerve trunk in the subcutaneous fat shows a small perineural epithelioid and round-cell lesion. Its nerve sheath is invaded by monocytes, and in the serial sections a capillary with proliferating endothelium is just within the nerve sheath, with several monocytes and epithelioid cells infiltrating the nerve bundle (slides 30 and 31). A small nerve in the deeper corium, which is ensheathed by an epithelioid lesion, shows similar invasive changes by the epithelioid cells (slide 20). One acid-fast bacillus in a lesion close to a smooth hair muscle (slide 17. section 2).

Protocol.—Born January 24, 1936. Separated from parents at 14 months. Never found positive for M. lepræ in smears.

Previous skin blemishes and relation to probable lesions:

- (C) May 26, 1936. A few pin-head subsiding vesicopapules on trunk and extremities. Dry seborrhœa anterosuperiorly on scalp.
- (C) December 4, 1936. Healing minute scables on feet. Pin-point or larger reddish vesicopapules anteriorly on upper thighs and abdomen. A few pin-point vesicopapules on cheeks.
- (C) February 2, 1937. All over face, trunk, and extremities are pinhead to large-pea-sized, rounded, crusted eruptions, surrounded by a zone of slight inflammation.

Separated from parents March 20, 1937, at 14 months.

- (N) April 1, 1937. A number of drying scabies on hands, toes, and left plantar. A few larger pin-head or larger than pin-head vesicopapules on abdomen. Small purplish scars on buttocks.
- (N) August 4, 1937. Subsiding pea-sized, reddish wheals medially on right arm and medially below left knee. Drying vesicopapule medially on upper right buttock and medially on lower left buttock.
  - (N) October 1, 1937. No definitely suspicious lesions.
- (N) December 3, 1937. (a) On upper left knee a small, shiny, pinkish, slightly elevated area, 5 mm in diameter, on each side roughly square. (b) Externally on left thigh, 1.5 inches above level of knee, a small, pinkish, very slightly elevated area, 6 mm in diameter. (This lesion biopsied March 25, 1938.) (c) Medially on upper left knee a small, similar area, not as pinkish as that on upper left knee. (d) Externally on middle of right thigh a small, pale pinkish, elevated, wheallike area, without red areola.
- (N) February 4, 1938. Externally on lower left thigh a small, pale, criny area, about 8 mm by 6 mm. Other lesions, mentioned above, December 3, still present.
  - (N) March 28, 1938; probable lesions:
- (a) Anteriorly on middle of left thigh a pale pinkish area, very slightly elevated, about 4 mm in diameter, may be a scar.
- (b) Externally on lower left thigh, about 1.5 inches above knee level, a pinkish, slightly elevated, rough, slightly indurated area, also hypopigmented, 8 mm in diameter, with pin-point, purplish areas on its surface (biopsied).
- (c) On upper left knee a similar, pale, slightly thickened area, 11 mm in diameter, with a central minute papule.
- (d) Externally on upper right thigh a small, hypopigmented, not distinctly elevated area, 5 mm in diameter, with a purplish, pin-point spot in the central portion.

Comment.—Previous skin blemishes were vesicopapules on extremities, in May, 1936; vesicopapules anteriorly on upper thighs, December, 1936; pinhead to large pea-sized, crusted eruptions on extremities, in February, 1937; one lesion, observed March 28, 1938, anteriorly on middle of left thigh, probably a scar.

CARE 12. GRE. AMA., 1 year and 10½ months on day of biopsy. Posterolaterally on right thigh. First noted February 19, 1938, as a pinkish elerated area about 2½ mm in diameter, surrounded by a faint, narrow, pale halo. March 8 it was still a pinkish flattened papule, but slightly larger, about 5 by 3 mm, still surrounded by a faint, pale halo. March 29, 1938, when it was biopsied, it was a deep-pink papule, 5 mm in diameter. Incised for smear taking once only, March 8, 1938; negative for acid-fast bacilli.

Histological.—Sections show a deep scar, 2 mm long (incision of March 8. 1938) near one end and extending through two-thirds of the depth of the corium. Embedded in the scar are small foci of epithelioid and round-cell collections. Beneath the scar and to one side close to it are medium-sized and small epithelioid and tuberculoid foci with a few Langhans's giant cells in relation to hairshafts, blood vessels, a small nerve trunk, and sweat glands. The papillary layer to one side close to the scar shows a narrow stretch of epithelioid lesion in close contact with the germinal epithelium (slide 25) causing fraying of the basal cytoplasm of their cells in places and one or two monocytes invading the deeper epidermis (Plate 1, fig. 4), In the aniline-blue sections a small nerve trunk in the deeper corium ensheathed with epithelioid and cut longitudinally shows a local swelling at one point along its course, with separation and fraying of its delicate, blue-staining, wavy connective tissue (slide 7). Other small nerve trunks in cross section show no appreciable changes, although they are ensheathed by epithelioid or tuberculoid lesions. Border of lesion appears to end rather abruptly, except for slight perivascular round cells in the papillary layer toward the normal skin. Two atypical acid-fast granular bacillary forms (?) found in a superficial lesion (slide 23, sections 1 and 2).

Protocol.—Born May 9, 1936. Separated from parents at 12½ months. Never found positive in smears.

Previous skin blemishes and relation to probable lesions:

- (C) July 29, 1936. One small wheal externally above right knee.
- (C) December 4, 1936. A number of pin-point to pea-sized reddish vesico-papules on upper back.
- (C) February 2, 1937. Many drying minute vesicopapules on feet and lower legs. Above and below right cubital fossa are patches of dark-red, thickened, closely set vesicopapules.
  - (C) April 3, 1\(\gamma\)37. Minute fading pale scars on chest. Separated from parents, May 29, 1937, at 12\(\frac{1}{2}\) months.
- (N) June 1, 1937. Fairly numerous fresh, dark-brown scars and some healing lesions, apparently of scabies, on legs, thighs, and buttocks, fewer on upper extremities.
- (N) February 19, 1938. On middle right buttock a deep-pink papule, about 3 by 2 mm. Posterolaterally on middle of right thigh a pinkish, slightly elevated area, about 2.5 mm in diameter, surrounded by a faint, narrow, pale halo (biopsied March 29, 1938). A hazy pale area medially on upper left buttock, about 50 by 20 mm. A hazy pale area anteriorly on upper right leg, slightly shiny and with a very slightly elevated center, the whole 10 by 5 mm. Externally on upper right leg two pin-point, reddish papules, slightly shiny, surrounded by a very faint pale halo. Medially on dependent portion of right buttock a pale-pinkish, slightly elevated area, 2.5 by 2 mm.

#### March 28, 1938; probable lesions:

- (a) On middle of right buttock, a 4-mm deep-pink papule.
- (b) A similar lesion on dependent portion of right buttock, 3 mm.
- (c) A similar lesion, posterolaterally on right thigh, 5 mm (biopsied).

(d) Externally on upper right leg two similar pale-pinkish papules, each 3 mm in diameter.

Comment.—Previous skin blemishes were fresh, dark-brown scars and some healing scabies lesions on legs, thighs, and buttocks, June 1, 1937.

Case 13; Lau. Pun., 2 years and 7 months on day of biopsy. Posteromedially on left elbow. First observed February 26, 1938, as a small, shiny, rather rough, hypopigmented area, about 1 cm in diameter, not elevated, with apparently slightly hypertrophied hair follicles. March 31, 1938, this slightly depigmented "goose-flesh" area was still distinct, about 10 mm in diameter, with irregular, slightly granular borders and apparently very minute daughter lesions, slightly hypopigmented near the borders. Biopsied on this day. Incised for smear taking only on day of biopsy, negative. Positive (+) for M. lepræ in the right ear lobe, upper portion, August 4, 1937, 7% months before biopsy of left elbow lesion.

Histological.—Serial sections show no histological evidence of scarring. In the papillary layer are many small epithelioid and tuberculoid lesions close to the epidermis, some of them in close contact with the germinal epithelium, causing fraying of the basal cytoplasm of some cells and infiltration with a few monocytes (slides 15 and 25). In the subpapillary and reticular layers, including the superficial layer of subcutaneous fat, are small and medium-sized epithelioid and tuberculoid lesions, with rare Langhans's giant cells around hairshafts, small blood vessels, and sweat glands. One smooth hair muscle is split and infiltrated by a tuberculoid lesion. Two small, thin nerves are found ensheathed in two tuberculoid lesions close to the subcutis (slides 35, 36, and 37) one showing evidence of compression at one point and the other infiltration and splitting of the nerve trunk by the lesions. One acid-fast bacillus in a very superficially located lesion (slide 14, section 4).

Protocol.—Born August 24, 1935. Separated from parents at 14½ months. Found positive (+) in smears for M. lepræ in the upper portion of the right ear lobe, August 4, 1937.

Previous skin blemishes and relation to probable lesions:

- (C) October 26, 1935. Papular eruptions, more or less diffuse anteriorly and posteriorly on trunk. A few on extremities and back of neck.
- (C) December 17, 1935. Fairly numerous, very minute vesicopapules on chest, extremities, abdomen, and middle of back; also a number of dark-brown drying vesicopapules on extremities. Minute dry vesicopapules, or pustules, on feet and around ankles.
- (C) February 14, 1936. Fresh scabies lesions on feet; minute papulovesicles on abdomen and back. A few scabies lesions on hands. Reddish indurated areas at both antihelices.
- (C) July 29, 1936. Feet and ankles dirty due to numerous pigmented scars. Lower legs with numerous, brownish, minute, subsiding vesicopapules, fewer similar lesions on thighs.
- (C) September 23, 1936. Extensive, dark, minute scars on buttocks, thighs, legs, and feet. On legs a few minute papulo-vesicular eruptions, a few also at interscapular region.

Separated from parents November 14, 1936, at 142 months.

(N) December 3, 1936. Numerous fading, minute, dark-brown scars and subsiding vesicopapules on thighs and legs. Two minute, crusted, healing

eruptions on lower right buttocks. A few pin-head vesicopapules on penis and scrotum.

- (N) February 1, 1937. A number of healing scabies around ankles.
- (N) June 1, 1937. On upper margin of right ear lobe an oval reddish area, about 6 by 3 mm, apparently soft, not eroded.
- (N) August 4, 1937. Above lesion, on right ear lobe, prominent, distinctly reddish, but soft, positive (+) for M. lepræ in smears.

A number of pale, depigmented, small areas with rough granular borders and granulations even of the surface, varying in size from 1 cm to 4 by 1.5 cm, located—

(a) On left side of chest; (b) above and 1 inch below left major trochanter; (c) on dependent portion of right buttock, and 1.5 inches below it; (d) externally over middle of right leg; (e) posterolaterally over middle of left leg; and (f) anterolaterally over upper part of right forearm. On left lumbar a hazy pale area with prominent follicles, about 2 inches in diameter.

Lesion c, d, e, and f, above, are suspected to be tuberculoid lesions.

Child returned to parents in the Colony August 5, 1937, as a positive case from the right ear lobe.

- (C) October 1, 1937. Ulcerated scabies and dark scars on feet, legs, and knees. A few pin-head vesicopapules on ulnar side of hands and back. Previous depigmented areas of August 4 still distinct and still somewhat granular, others apparently smoother.
- (C) December 9, 1937. Posteromedially on middle of right thigh a pale area, 1 cm in diameter, showing pin-head, flattened papules near its upper margin. Positive lesion on right ear lobe, 10 by 6 mm, distinctly red and rounded. A number of brown and pale scars on hands, wrists, buttocks, posteriorly on thighs and legs, upper back, externally on left arm. Fairly numerous fresh scars and some drying pea-sized eruptions, probably scabies, on dorsum of feet, anteriorly on ankles, medially above left knee, and posteriorly on right/ankle.
- (C) February 7, 1938. A number of pin-head, brownish, drying vesico-pustules, anteriorly on thighs, abdomen, chest, anteriorly on arms, axillary and dorsal. Previous lesion posteromedially on middle of right thigh is a pale area, 13 by 10 mm, showing a linear scar from smear taking. Positive lesion on right ear lobe still reddish, shiny, 15 by 7 mm.
- (C) February 26, 1938. Near left elbow, posteromedial aspect, a small, shiny, rather rough, hypopigmented area, 1 cm in diameter, not elevated, with apparently slightly hypertrophied hair follicles.
  - (C) March 31, 1938; positive and probable lesions:
- (a) Positive lesion on upper right ear lobe seems smaller and more like a wheal, 8 by 6 mm, slightly reddish.
- (b) Slightly depigmented area posteromedially on middle of right thigh, 15 by 10 mm, still fairly distinct but not showing distinct goose-flesh surface.
- (c) Slightly depigmented goose-flesh area posteromedially on left elbow, still distinct, 10 mm in diameter, with irregular, slightly granular borders and apparently very minute daughter lesions, slightly hypopigmented near borders (biopsied on this day). No other definitely suspicious lesions.

Comment.—Previous skin blemishes were a few papular eruptions on extremities in October, 1935, numerous minute vesicopapules and dark-

brown drying similar lesions on extremities in December, 1935, reddish indurated areas on antihelices in February, 1936, subsiding vesicopapules on thighs in July, 1936, extensive minute scars on thighs in September, 1936, numerous fading, dark-brown scars and subsiding vesicopapules on thighs in December, 1936, pin-head vesicopapules on forearms in October, 1937, brown and pale scars posteriorly on thighs and externally on left arms in December, 1937, and pin-head, brownish, drying vesicopustules anteriorly on thighs and anteriorly on arms in February, 1938.

Because of the unusual site of the positive lesion in the upper right ear lobe, a personal inquiry from the parents of the child was made. The mother said she remembered that the child had scabies of the ears at the age of 6 months. This report tallies with the observations of reddish indurated areas on both antihelices, February 14, 1936, when the child was about 6 months old. The father, quite intelligent, declared that the child had had scabies behind the right ear, very close to the site of the positive lesion on the upper margin of the right ear lobe.

Case 14. Pre. Par., 3 years and 4 months on the day of biopsy. Above left popliteal. First noted April 7, 1937, 11 months before biopsy, as a small, bean-sized, pale-pinkish, slightly raised, shiny area, a scar, according to the father. June 4, 1937, this pale pinkish area, suspected of being a "scar," was still quite distinct and appeared different from other scars in the vicinity. August 6, 1937, it was distinct, about 1 cm in diameter. October 1, 1937, it was still distinct, with a pinkish-brown center. December 6, 1937, it appeared slightly larger but less elevated, with a brownish center. February 7, 1938, it was a pale area, 10 by 8 mm. Biopsy March 12, 1938. Incised for smear taking August 6, 1937, and October 11, 1937; negative for acid-fast bacilli in both instances.

Histological.—Biopsied specimen very small. Serial sections show no evidence of scarring. At one end the papillary layer shows some perivascular round-cell infiltration, mixed with several large monocytes apparently undergoing very early epithelioid changes. None in contact with epidermis. In the subpapillary and reticular layers are three medium-sized epithelioid and tuberculoid lesions in relation to a hairshaft and a group of coils of sweat glands. Close to the subcutis (slide 10) a small nerve trunk with perineural epithelioid infiltration shows involvement of the epineural sheath, and one large monocyte with a homogenous eosin-staining cytoplasm among the nerve fibrils. No scar of incision from smear taking was found, possibly not included in the biopsied specimen. Acid-fast bacilli could not be demonstrated in any of the large number of sections examined.

Comment.—This lesion was suspected of being a scar 11 months before biopsy. The absence of any histological evidence of scarring may be due to regeneration of the elastic tissue, or noninclusion of the scarred area in the biopsy, which was very small.

Protocol.—Born November 18, 1934. Separated from parents at 11 months, October 14, 1935. Released to relatives January 14, 1936. Returned to Culion from San Lazaro Hospital, Manila, said to have been found positive in smears for M. lepræ in the sacral region January 21, 1936, at the age of 14 months. Biopsy report of positive lesion at San Lazaro: "Perivascular infiltration. Cannot be examined for M. lepræ, due to accidental use of Xylol." Returned to parents in the Colony March 11,

1936. Never been found positive in smears after return to Colony, from March, 1936, up to March, 1938.

Previous skin blemishes and relation to probable lesions:

- (C) May 10, 1935. Anteriorly on thighs many hazy pale areas, on left thigh one lesion with a small brownish center, another lower down with a red wheal at center.
- (C) August 21, 1935. Cheeks slightly rough, due to a few minute, papular irritations. Ill-defined pale areas anteriorly on thighs and anterolaterally on legs, the latter very slightly shiny.

Separated from parents October 14, 1935, at 11 months.

- (N) October 28, 1935. A few moist, ulcerated eruptions on scalp, one on right cheek.
- (N) December 4, 1935. Healing, crusted eruptions above right buttock and over occipital. A few active scabies on right small finger.
- (N) December 14, 1935. Dark, reddish-brown, corn-sized scar on middle of right cheek.

Returned to parents in Colony March 11, 1936, found positive in the right sacral in San Lazaro Hospital, Manila, January 21, 1936 (see first paragraph under protocol, above).

- (C) March 14, 1936. Fairly numerous, scattered, pin-head vesicopapules on buttocks, back, and extremities, and active scabies lesions on feet, lower legs, and posteriorly on left thigh.
- (C) April 10, 1936. Extensive, scaling, superficially ulcerated eruptions on legs, less marked on upper abdomen, a few anteriorly on right thigh, cheeks, back, and right hand.
- (C) May 28, 1936. Fairly numerous drying scabies on feet, ankles, and hands. Extensive brown scars on legs. A pea-sized, red, eroded vesicopapule posteriorly on left thigh.
- (C) July 31, 1936. A few pin-head to pea-sized vesicopustules posteriorly on thighs, probably scabies.
- (C) September 22, 1936. Some thickened, dark scars on inner side of thighs and below buttocks.
- (C) April 7, 1937. Numerous dark-brown scars on trunk, thighs, but-tocks, and legs. Above left knee and externally on middle of left thigh and posteriorly on lower left thigh (above left popliteal) three small, pale, pinkish, slightly raised, shiny areas, scars according to the father.
- (C) June 4, 1937. Above suspected "scars" appear different from other scars in the vicinity, being slightly pinkish, slightly thickened, and slightly shiny.
- (C) August 6, 1937. Pale pinkish areas on the left thigh are distinct, 1 to 1.5 cm in diameter, slightly raised.
- (C) October 1, 1937. Previous pale areas posteriorly on lower left thigh, externally on left thigh, and above left knee still distinct, with pinkish-brown centers; the first two with slightly elevated centers. They do not seem to be increasing in size, but a pale halo adjoins lateral portion of area above left knee and upper portion of area externally on left thigh. A number of pea-sized or smaller papulopustules anteriorly on thighs, left elbow, and a few on buttocks.
- (C) December 6, 1937. Previous three pale areas appear slightly larger but less elevated and now have brownish centers. No new suspicious le-

sions. A number of pea- to bean-sized, drying, crusted eruptions, posteriorly on thighs, buttocks, sacral, left forearm, medially on right arm.

Positive and probable lesions:

(a) January 21, 1936, on right sacral a superficial brownish area surrounded by a pale areola. (Found positive in smears, and biopsied at San Lazaro Hospital.)

February 7, 1938, the following probable lesions were noted:

- (a) A pale area above left popliteal (posteriorly on lower left thigh), 10 by 8 mm, slightly raised. (Biopsied in Culion, March 12, 1938.)
- (b) Externally on middle of left thigh a similar pale area, 10 mm in diameter, slightly raised.
  - (c) Above left knee a similar area, 25 by 10 mm, also slightly raised.
  - (d) A pale area, 8 mm in diameter, medially over left elbow.

Comment.—Previous skin blemishes were healing, crusted eruptions above right buttock December 4, 1935; numerous vesicopapules on extremities, and active scabies lesions posteriorly on left thigh in March, 1936; a peasized, eroded vesicopapule posteriorly on left thigh in May, 1936; vesicopapules posteriorly on thighs in July, 1936; thickened dark scars on inner thighs and below buttocks in September, 1936; pale pinkish suspected scars above left knee, externally on middle of left thigh, and posteriorly on lower left thigh in April, 1937; papulopustules anteriorly on thighs and left elbow in October, 1937; and crusted eruptions posteriorly on thighs and left forearm in December, 1937.

#### DISCUSSION

Of the fourteen biopsied cases here reported, the lesions as they were first observed clinically were as follows:

In one, a hazy pale area, about 1 cm in diameter,  $3\frac{1}{2}$  months before biopsy, which became a smaller, hypopigmented, pink, slightly raised area, 5 by 4 mm, at the time of biopsy (case 2).

In two, a pale area, 25 by 15 mm, slightly granular, in the lower portion (case 4), and a small, shiny, rather rough, hypopigmented area, about 10 mm in diameter, not elevated, with apparently slightly hypertrophied follicles (case 13).

In three, scarlike, two of them being definitely indurated scars (cases 3 and 10), while one was a small, pale pinkish, slightly raised, shiny area (case 14).

In eight, flat, wheallike, in three of them as pinkish, slightly shiny papules, from pin-head to 3 mm in diameter (cases 6, 7, and 8) and in five as small, pale pinkish, or pinkish shiny, thickened, or slightly elevated areas, varying from 2.5 mm in diameter to 7 by 5 mm (cases 1, 5, 9, 11, and 12).

Of the above fourteen cases, in eight the lesions were definitely papular or thickened when first observed (cases 1, 5, 6, 7, 8, 9, 11, and 12), at periods varying from 8 days to 3 months and 3

days before biopsy. In a ninth case (case 2) the pink papule, 5 by 4 mm, was preceded by a hazy pale area  $3\frac{1}{2}$  months before biopsy, and in a tenth case (case 14) it was scarlike. In four of the above cases (6, 7, 8, and 9) and in one indurated scar (case 10), three were found positive (++) to (+++) for M. leprx in smears on the day the lesions were first discovered (cases 6, 7, and 10), one (case 8) (+) on the day of the biopsy, and one (case 9) was found positive (+) in the remaining half of the lesion left in the child for further clinical observation, a little over 2 months after biopsy.

As already mentioned in the first part of this paper, Lara and de Vera(9, 10) were apparently the first to point out the occurrence of an unrecognized characteristic leprotic papule, usually positive for M. lepræ, occurring very early in infants of leprous parents that have become declared lepers. Chiyuto (2,3) has mentioned similar lesions, but no smears were made from the lesions of his cases, and in five biopsies, which were apparently histologically tuberculoid lesions, no acid-fast bacilli were demonstrated. Wade, (26, 27) in a study of South African tuberculoid material, was able to demonstrate acid-fast bacilli only in what he considered reacting cases, but none in the nonreacting cases. On the other hand, Lie(11) claims to have been able to demonstrate the bacillus in all ten South African specimens he examined, four of which were considered typical examples of tuberculoid leprosy, and suggested that the search for the bacilli should be made patiently, carefully, and systematically.

Concerning the presence of acid-fast bacilli in the sections from our cases (Plate 3, figs. 10 and 11) a special effort was made to find them, at times a whole day being employed in the examination of one case. The findings were as follows: in four (cases 6, 7, 9, and 10) they were from several to many and easily found; in seven (cases 1, 3, 4, 5, 8, 11, and 13) they were scanty, or very scanty, and only one or two were found in from several to many sections examined; in two (cases 2 and 12) doubtful acid-fast bacilli were found; and in one (case 14) no acid-fast bacilli could be demonstrated. As M. lepræ presents very variable forms in the tissue sections, as has been observed in case 10, the two doubtful cases might also have been declared positive, except for the absence of other definite bacillary forms in the tissues. One interesting finding is the occurrence of one acidfast bacillus in a focus of round-cell collection in the section showing only perivascular round-cell infiltration (case 4).

Histologically, of these fourteen cases, eleven (cases 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14) showed definite epithelioid and tuberculoid changes (Plate 3, figs. 8 and 9); in two (cases 2 and 3) some of the large monocytes in the lesions showed early epithelioid changes (Plate 1, fig. 1); and in one (case 4) only perivascular round-cell infiltrations mixed with a few monocytes were found. An interesting finding in the case with perivascular round-cell infiltrations is the splitting and infiltration of the smooth hair-muscle bundles by the infiltrating cells (Plate 1, fig. 2). In all the tuberculoid sections the smooth hair muscles were definitely invaded, producing splitting and isolation of groups of the muscle fibers.

The tuberculoid lesions were invariably distributed around blood vessels, hairshafts, and smooth hair muscles, sweat-gland ducts, and coils and small nerves. In most the lesion foci were small or medium-sized, while in one (case 5) they were fairly extensive, and in another (case 10) extensive and closely set (Plate 3, fig. 8). In the papillary layer the lesions in most of the cases consisted of a thin layer of epithelioid or tuberculoid lesions side by side, from which downward prolongations of the lesions into the deeper corium could be traced in serial sections.

In eleven cases, ten of which were definitely tuberculoid lesions and one showed only beginning epithelioid changes of the large monocytes, the most superficially located lesions were found to be in close contact with the germinal epithelium, seemingly producing fraying of the basal cytoplasm of their cells. In five of these (cases 1, 5, 7, 12, and 13) besides fraying of the basal cytoplasm of the germinal epithelium, there was also concomitant invasion of the deeper layers of the epidermis by a few monocytes (Plate 1, figs. 3 and 4). Wade(26) has already described similar changes in tuberculoid leprosy from South African material. To verify this point from Philippine material, twelve sections from tuberculoid leprosy cases in Culion on file in our laboratory were examined and showed similar changes.

Concerning the nerves in the sections, which are ordinarily surrounded or ensheathed by epithelioid or tuberculoid lesions, no apparent involvement was found in three (cases 2, 3, and 4), but in the other eleven cases the surrounding epithelioid or tuberculoid lesions have been found apparently invading their epineural sheaths in three (cases 7, 8, and 10), and actual invasion of the nerve fibers, either by monocytes or epithelioid cells in eight (cases 1, 5, 6, 9, 11, 12, 13, and 14). In five of these eight cases

from one to several monocytes were found infiltrating the nerve fibers (Plate 2, fig. 7); in one (case 5) from partial to complete tuberculoid infiltration of the nerve trunk (Plate 2, figs. 5 and 6); and in two (cases 12 and 13) swelling or infiltration with separation of the delicate blue-staining connective tissue of the nerves. These changes in the nerves due to invasion by the perineural lesions, however, were not uniform, being found only at certain levels in the serial sections examined. One of us (C.B.L.) previously observed in a few instances a lack of feeling on the part of the child when some of the definitely suspicious early papular lesions were first incised for the bacteriological examinations. Wade(26) has mentioned that as a rule the tuberculoid process invades only the outer sheaths when it is invasive at all. Muir and Chatterji(17) and Lowe(12) have shown the fine nerve branches in "tuberculoid" macules infiltrated with "epithelioid," Grieco(7) and Ermakova(4) have also shown invasion of the small cutaneous nerve trunks by lymphocytes and histiocytic, or epithelioid cells.

Special efforts were made also to determine the presence of scars in the section. The incisions for smear taking made on the lesions previous to biopsy interfered somewhat in the microscopic examinations. Of the three lesions (cases 1, 5, and 13) incised only once on the day of biopsy, no scars were found in the sections, and of the eleven that had been incised from 20 days to 7 months before biopsy, definite scars attributable to the incisions were found in nine, while in two (cases 10 and 14) these incision scars were not found, possibly not being included in the biopsies. Two of the lesions (cases 3 and 10) were originally observed clinically as definite scars, or scarlike, and these two cases showed histologically the original scars. besides the incision scar in one (case 3). In the third case, which was suspected of being a "scar" (case 14), no scar could be demonstrated, possibly because of the very small size of the specimen excised.

The finding of three lesions, two of them developing beneath definite scars and one scarlike out of these 14 biopsied cases, leads to the suspicion that previous skin blemishes may possibly play a rôle in the development of these very precocious lesions. The protocols show that two other lesions not biopsied, one anteromedially on the lower right thigh (case 10) and one anteriorly on the middle of the left thigh (case 11) were also suspected of being "scars." In the two lesions biopsied (cases 3 and 10),

the scars were so definite clinically that one cannot avoid suspecting the development of the lesions as a result of the breach of surface continuity of the skin. It is also, however, possible that in these cases the development has been merely fortuitous or accidental, since in many other very early probable lesions no apparent scars were observed when they were first discovered. In these cases, however, where there is no gross evidence of scars, a very superficial previous blemish of the skin may possibly have left no recognizable traces of scarring. Lara and de Vera(9) have already pointed out that various kinds of known nonleprotic skin affections, like prickly heat, eczema, dermatitis, insect bites, scabies, bruises, and other forms of injury, if only superficially involving the skin surface, may leave no scars at all upon healing, but instead only hazy, pale, or depigmented areas.

Analyzing the protocols of the cases here reported for previous skin blemishes, by region, in relation to the sites of both biopsied and not biopsied positive and probable "leprotic" lesions, it has been found that of the 42 regions showing positive or probable leprotic lesions (Table 1), 38 regions showed previous skin blemishes in the form of reddish wheals, vesicopapules of from pin-head to pea-sized, scabieslike lesions, minute pale scars, fresh scars, brown thickened scars, papular eruptions, ulcers—probably scabies, extensive scabies, crusted eruptions, and papulopustules. Only in four regions showing lesions was there no mention in the protocols of a previous skin blemish. Ten regions showed two lesions each in seven cases (cases 1, 2, 5, 10, 11, 12, and 14) three times on the right thigh, three times in the left thigh, and once each on the right knee, left knee, right leg, and right buttock, respectively (Table 1). In Table 2 is shown the regional incidence of localization and the number of lesions in them —fourteen times with 20 lesions in the thighs, six times with 8 lesions in the knees, six times with 7 lesions in the legs, four times with 5 lesions in the buttocks, three times with 3 lesions in the left elbows, two times each with 2 lesions in the right forearm, arms, and cheeks, and only once each with lesion in the right ear lobe, lower lumbar, and right sacral.

Table 2 shows that the larger number of the lesions are mainly on the exposed, or partially exposed regions of the body surface, as the thighs, knees, buttocks, elbows, forearms, arms and cheeks. Only in three instances were the lesions localized in the ear lobe, lower lumbar, and right sacral, one lesion in each

case. Although 13 of these 14 children showed previous skin blemishes on the trunk, only two lesions (lower lumbar and right sacral) developed in the trunk, compared with the other exposed regions of the body. This may indicate that the clothing worn by the child to some extent helped in protecting the covered portions of the body from the infection.

Marchoux and Sorel,(16) working on rat leprosy, were unable to produce infection in eight 8-day-old rats whose skins were still soft by painting the apparently intact skins over a large area with bacillary suspensions of rat leprosy, and concluded that the intact skin of young rats resists the penetration of the germs. On the other hand, Sakurai,(23, 24, 25) in a tabulated summary of experimental studies on the penetration of lepra bacilli through the skin in one neural case of leprosy, and in rabbits and mice, has shown that there was most penetration in injured skin, next in skin from which the hair had been pulled, third through the normal skin when the emulsion was rubbed in, fourth through the mucous membranes of the eyelid and nostril, and least in healthy skin without friction. In his lone human experiment(23), a few bacilli, or globi, were found in a minute chap of the skin that could not be seen with the naked eye.

The multiple lesions varying from 2 to 7 in these fourteen children (Table 1), except two (cases 3 and 8) that apparently had only one lesion each in the legs, suggest multiple sites of infection through the skin, apparently favored by previous skin disease. This possibility is definitely suggested in one positive, oval, reddish lesion (case 13) of unusual location in the right ear, which had a history, according to the father, of scabies behind the right ear close to the site of the positive lesion in the upper border of the ear lobe.

It might also be argued and assumed that the recognizable early lesions in these cases had been latent infections (possibly lying latent in the deep organs or skin) which became manifest at these unusual early ages. This explanation is possible, but in an unpublished histological study on the presence of leprotic lesions and acid-fast bacilli of the deep organs (spleen, liver, lymph nodes, and nerves), by both the paraffin and frozen methods of sectioning, of 58 necropsied children in Culion, all below 2 years of age, none was found positive. (21) Similar unpublished histological examinations (21) of "hazy depigmented areas" of the skin, of the type first described by Chiyuto (1) in

TABLE 1.—Regional distribution of positive and probable lesions and previous skin blemishes.

Previous skin lesions.	Kind.	None.	Scabieslike lesions and scars.	Vesicopapules.	Numerous pale and brownish scars.	Vesicopapular eruptions.	Minute pale scars, probably scabies.	Do.	None.	Lesion actual scar, probably scabies.	Scars and vesicopapules, probably scabies.	Do.	Fresh scars, probably scabies.	None.	Vesicopapules.	Do.	Reddish wheals.	Dark-brown spots and scars, probably scabies.	Dark-brown spots, probably subsiding wheals.	Papular eruptions and scar.	Scars, ulcers, probably scabies.	Vesicopapules and ulcers, probably scabies.	Reddish wheals, vesicopapules, and scars.	None.	Sears, probably scabies.	Extensive scables and scars.	Do.	Do.	Do.	Do.
1	Presence or absence.	1	+	+	+ -	+	+	+	23	+	+	+	+	es	+	+	+	+	+	+	+	+	+	 	+	+	+	+	+	+
Number of	and probable lesions.	62	1	67			23	-	-	1	1	-	red	14	1	d 22	7	91	-	41	T p	<del></del>	4.1	T 0	m		g 7	**	62	d 1
	Region.	Right thigh	Right leg	Left thigh b	Lower lumbar	Left cheek	Right thigh b	Right buttock	Left buttock	Left leg b	Right thigh b	Left thigh	Left leg	Above left elbow b	Left knee	Right knee	Right cheek	Right thigh b.	Right forearm	Right arm b	Right thigh	Left buttock	Left leg b	Right knee b	Right forearm	Left arm.	Right thigh	Left thigh	Left knee	Right leg b.
Sex.	Female.	-		+		_		7	آب	1	-	, 			Ì	_	,	1		,	1		1	_						
S.	Male.							+		+	+				+			+			+		+	+				+		
9	e. Months.			4				63		88	11,				115			6			co		00	73				co		
Age.	Years.			83				27			***							-			1		C3					22		
	Name.			Jos. Lun				Moi. Mif.		Bue. Mia.	Sul. Dis.				Rod. Abe.			Ern. Vib.			Epi. Gal.		Edi. Uro.	Ans. Man.				Ren. Tab.		
	Сазе.							N		00	- - - - - - - - - - - - - - - - - - -				16	>		9	_	-	7	_	00	ø.	-			10		

Vesicopapules, crusted eruptions, scars. Reddish wheals. Vesicopapules, crusted eruptions.	Scars, probably scabies. Do. Do.	Indurated areas, scabies according to the father. Vesicopapules, scars, probably scabies. Do.	Crusted eruptions. Vesicopapules, scars, probably scapies. Vesicopapules, probably scapies. Vesicopapules, probably scabies. Vesicopapules, propulopustules, probably scabies.		Positive 2 months after biopsy.
+++	+++	+++	++++	38+4-	
C7	01 H 02		- 01	38	
		TO	Срик	10	938.
Left thigh bRift kneeRight thigh	Right buttock Right thigh b Right leg.	Right ear lobe	Right sacral Left thigh b Left knec	42	<sup>c</sup> Positive June 29, 1938. <sup>d</sup> Positive.
+	+	1	+	4	
1		+		10	=
67	10}	_	4	2 0 0 0	n protoc
Ø	-	01	ಣ		down i
Vir. Ren.	Gre. Ama	Lau, Pun,	Pre. Par.	Total	<sup>a</sup> None noted down in protocol. <sup>b</sup> Biopsied.
11	12	13	14		

TABLE 2.—Regional incidence and distribution of positive and probable lesions in the 14 cases examined.

	Incidence	Number of lesions.			
Region.	of regio al localiza- tion.	Total.	Positive in smears.		
1. Thighs	14	20	4		
2. Knees	6	8	2		
8. Legs	6	7	2		
4. Buttocks	4	5			
5. Elbows (left only)	3	3			
6. Forearms (right only)	2	2			
7. Arms	2	2	1		
8. Cheeks	2	2	1		
9. Right ear lobe	1	1	1		
10. Lower lumbar	1	1			
11. Right sacral	1	1	1 8		
Total 11 regions	42	52	12		

a Positive at San Lazaro Hospital.

20 necropsied Culion children, also under 2 years of age, showed no histological changes from similar normal control skin sections.

While penetration of the intact human skin by *M. lepræ* is possible, the presence and great frequency of skin diseases in these very young children whose surroundings may be considered as heavily permeated with leprosy bacilli, would certainly favor and facilitate implantation of the infection. Because of their multiple localization, and because of their tendency to show *M. lepræ* very early, or as soon as they have been discovered clinically, these early papular thickened lesions may be considered primary inoculation lesions, and may be compared to the primary focal lesions of tuberculosis in the lung parenchyma of children. (19) Their inconspicuousness, unless special efforts are made to find them, has already been mentioned by one of us. (8)

An ideal control for this study would be to prevent the development of skin diseases in a group of children living with their leprous parents. By close medical supervision and a careful daily check-up and treatment of all skin diseases that may develop both in the children and in their parents, it may be possible to bring up children absolutely free from any skin blemishes, to which is in part attributed their early infection. The difficulties in carrying out this suggestion become at once apparent in view of the extreme susceptibility of the tender skin of infants and young children to various types of nonleprotic affections.

# SUMMARY AND CONCLUSIONS

- 1. Fourteen very early "leprotic" lesions from fourteen very young children of lepers from 1 year and 3 months to 3 years and 3 months old were studied histologically.
- 2. In eight the lesions were clinically definitely papular or flat and wheallike when first observed; in a ninth case the papule was preceded by a hazy pale area; in one it was a pale area slightly granular in the lower portion; in one a rough hypopigmented area with apparently slightly hypertrophied follicles; and in three, indurated scars or scarlike areas.
- 3. Of the papular lesions, including one indurated scar, examined by the "scraped-incision" method, three were found positive for *M. lepræ* on the day the lesions were discovered, one on the day of the biopsy, and one in the remaining half of the lesion left in the child, a little over two months after biopsy. The others were negative.
- 4. In the skin sections, acid-fast bacilli have also been demonstrated from several to many in four cases, scanty or very scanty in seven cases, doubtful in two cases, and none in one case.
- 5. The significance of the non-acid-fast bacillary forms and non-acid-fast diptheroids and coccoids reported in smears and found in the sections, has not been established. As far as the present observations go, they are interpreted to be probably degenerated chromatin material of the numerous cells crowded in the lesions. The need for further investigations to determine their exact nature is mentioned.
- 6. Histologically, eleven showed definite epithelioid and tuberculoid changes, the lesions invariably distributed around blood vessels, hairshafts, smooth hair muscles, sweat-gland ducts and coils, and small nerves. In most the lesion foci were small or medium-sized, while in one (case 5) they were fairly extensive, and in another (case 10), extensive and closely set (Plate 3, fig. 8). Of the three showing no definite tuberculoid lesions, in two (cases 2 and 3), some of the large monocytes in the lesions showed beginning epithelioid changes (Plate 1, fig. 1) and in one (case 4) only perivascular round-cell infiltrations mixed with a few monocytes (Plate 1, fig. 2).
- 7. Interesting observations in the section showing only perivascular round-cell infiltrations (case 4) are recorded,—the infiltration and splitting apart of the smooth hair muscle bundles by the infiltrating cells (Plate 1, fig. 2) and the finding of an

acid-fast bacillus in a focus of round-cell collection. The latter biopsy was made from the center of a hazy pale area 28 by 30 mm, with minute papules chiefly along the borders.

- 8. In eleven cases, ten of which were definitely tuberculoid lesions and one showed only early epithelioid changes of the large monocytes, the most superficially located lesions were in close contact with the germinal epithelium, seemingly producing fraying of the basal cytoplasm of their cells and invasion of the same by a few monocytes (Plate 1, figs. 3 and 4).
- 9. Early involvement of the fine nerves in the lesions is apparent, as the epineural sheaths were found apparently involved in three cases, actual invasion of the nerve fibers by monocytes was found in five cases (Plate 2, fig. 7) swelling and infiltration of the nerves in two cases, and partial to complete tuberculoid infiltration of the nerve trunk in one case (Plate 2, figs. 5 and 6).
- 10. In the two lesions originally discovered as developing in definitely indurated scars, apparently of scabies, the original scars were identified histologically in the sections, while in the third, which was suspected of being a "scar," none could be demonstrated histologically, possibly because of the small size of the specimen excised.
- 11. Because of the multiple lesions, varying from 2 to 7, in these fourteen children, except in two which apparently had only one lesion each in the legs, the possible rôle of previous skin disease in favoring the implantation of the infection is discussed.
- 12. Although thirteen of these fourteen cases showed previous skin blemishes on the trunk, the rarity of the lesions in the trunk compared with the number of lesions found in the more exposed portions of the body (Table 2), is attributed to the clothing worn by the child, which may to some extent help in protecting the covered portions from the infection.
- 13. The very early papular thickened lesions studied, because of their multiple localization, inconspicuousness, and tendency to show *M. lepræ* very early, or as soon as they have been discovered clinically, may represent primary inoculation lesions comparable to the primary focal lesions of tuberculosis in the lung parenchyma of children.
- 14. The difficulties in carrying out a suggested control experiment are mentioned.

#### ACKNOWLEDGMENTS

Our thanks are due Dr. J. G. Samson, formerly in charge of the surgical work in Culion and at present Chief of the Leprosy Control Section, Manila, who obtained the biopsy material in this study, and to Dr. J. C. Manalang, of the Pathological Section, and Dr. H. W. Wade, of the Leonard Wood Memorial, for their courtesies in examining and verifying the acid-fast bacilli found in the several cases where only one or two, or doubtful bacillary forms have been found. Thanks are due also to Dr. J. M. Raymundo, Chief of the Culion Leper Colony, for loaning the services of Mr. Secundino Barraquero, formerly Technician and Artist of the Leonard Wood Memorial and at present Farm Adviser of the Colony, who took the microphotographs.

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# **ILLUSTRATIONS**

### PLATE 1

- Fig. 1. Large monocytes showing early epithelioid changes. No definite tuberculoid lesion was found in the serial sections of this case. (Moi. Miñ., case 2.) H. & E. stain.
  - 2. Smooth hair-muscle bundle infiltrated by round cells and a few large monocytes, causing splitting and isolation of groups of muscle fibers. In this case all the lesions in the section were perivascular round-cell infiltrations with a sprinkling of a few large monocytes. (Sul. Dia., case 4.) H. & E. stain.

Fraying of basal cytoplasm of germinal epithelium and infiltration
of the deeper layers of the epidermis with occasional monocytes.
(Rod. Abe., case 5.) H. & E. stain. A larger magnification of
similar changes is shown in fig. 4 (case 12).

 Fraying of basal cytoplasm of germinal epithelium and infiltration of the same with monocytes. (Gre. Ama., case 12.) H. & E. stain.

### PLATE 2

- Fig. 5. Partial tuberculoid infiltration of a medium-sized nerve trunk in the subcutis. (Rod. Abe., case 5.) H. & E. stain.
  - 6. Complete tuberculoid infiltration of the nerve trunk shown in fig. 5 at a deeper level in the serial sections. H. & E. stain. This lesion is best shown with Mallory's aniline-blue stain.
  - 7. Monocytes within the capsule of a small nerve trunk and epithelioid invasion of the epineural sheath. (Ana. Man., case 9.)
    H. & E. stain.

### PLATE 3

- Fig. 8. A superficial scar beneath which are extensive and closely set apparently undisturbed epithelioid and tuberculoid lesions. Note dilated blood and lymph vessels below scaling epidermis. (Ren. Tab., case 10.) Voerhoff's elastic-tissue stain.
  - 9. Higher magnification of superficial scar in the immediate vicinity of the hairshaft shown in fig. 8, marked x. Epithelioid and tuberculoid lesions undisturbed, blood and lymph vessels dilated, and elastic fibers absent in the scarred area. (Case 10.) Elastic-tissue stain.
  - Two long solid forms of M. lepræ in tuberculoid lesion (case 10).
     Acid-fast stain.
  - 11. One segmented form of M. lepræ from the specimen shown in fig. 10. Acid-fast stain.



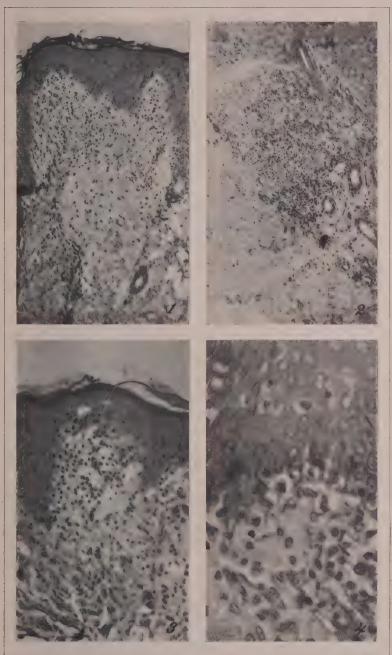
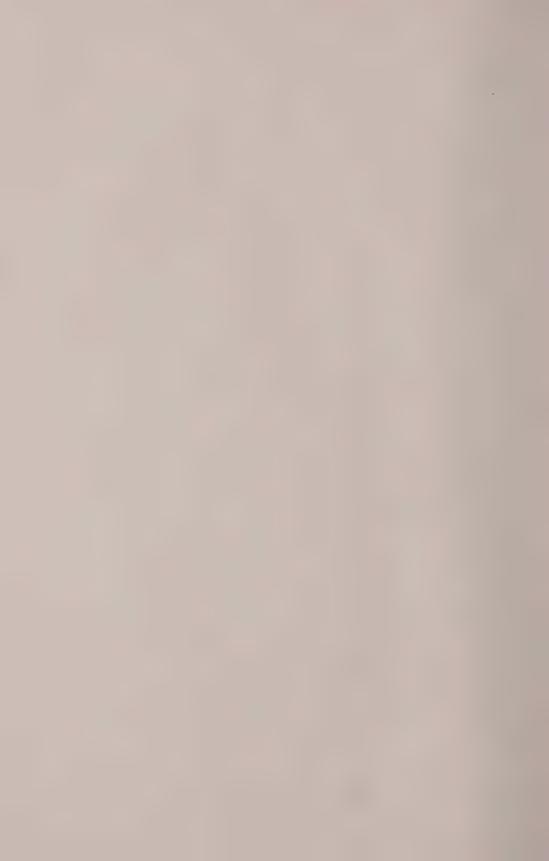


PLATE 1.



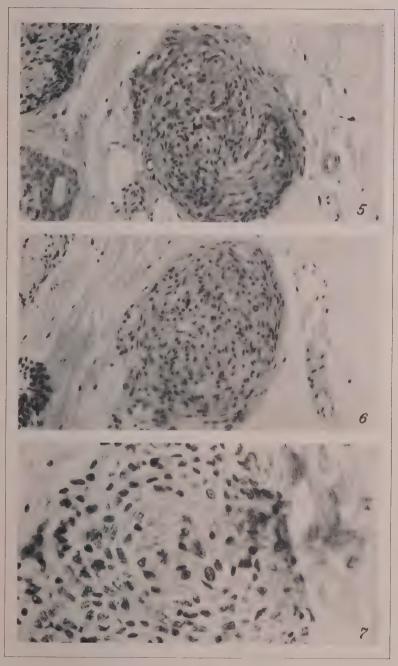
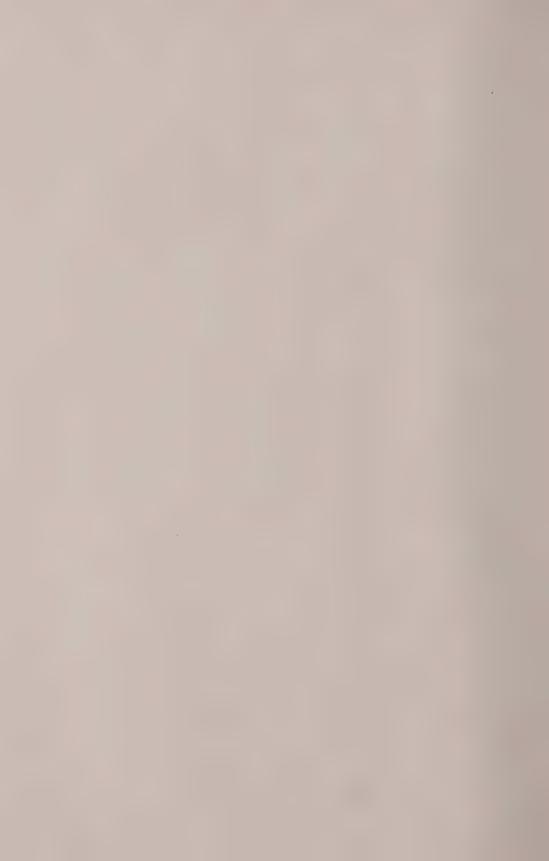


PLATE 2.



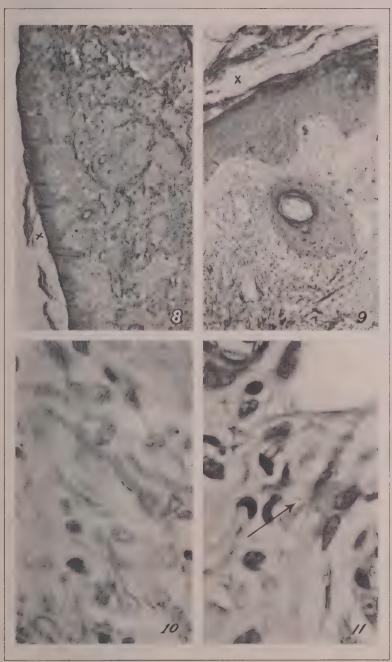


PLATE 3.



# PHARMACOLOGICAL STUDY OF QUARTERNARY ALKA-LOIDS AND FLUID EXTRACT OF PHAEANTHUS EBRACTEOLATUS (PRESL) MERRILL (KALIMATAS)<sup>1</sup>

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### TWELVE PLATES

Phaeanthus ebracteolatus (Presl) Merrill is a common and widely distributed plant in the Philippines. In Los Baños, Laguna Province, and in Bataan Province it is known as "kalimatas." The kalimatas bark used in the experiments reported in this paper, and a close picture of the trunk of Phaeanthus ebracteolatus, shown in Plate 1, were received from Mr. Mamerto D. Sulit, Forest Supervisor of the Bureau of Forestry, through the National Research Council of the Philippines. I wish to take this opportunity to express my heartiest thanks to Mr. Sulit and to Dr. P. Valenzuela, executive secretary of the National Research Council, for their valuable help. Sulit(3) includes in his article the following statement with regard to the plant:

Phaeanthus ebracteolatus (Presl) Merr. Family Anonaceæ

Common names: Alatáuan, banátan, banitan, banitang, dalinas, kalimatás, katinau, lanótan, lanótang-itim, lanután, oyoi (Tag.); langlañgás, takulan (Ilk.); marasigiát (Gad.); puropugai (Neg.); yamban (Zbl.).

A small to medium-sized tree reaching a height of 6 to 8 meters and a diameter of 20 cm or more with an open crown and more or less horizontal branching. The bole is regular in shape, with dark-gray bark. When cut the color of inside bark is yellowish-cream, varying in thickness from 8 to 10 mm depending upon the size of the tree, fibrous and bitter in taste.

The leaves are alternate, subchartaceous, sparsely hairy beneath, oblong to oblanceolate, upon 5 to 10 mm long fulvous petioles, obtuse or bluntly acute, broadly obtuse to rounded at the base, 10 to 15 cm long by one-third as wide.

<sup>1</sup>This paper covers a part of the work carried out under the grant-inaid of the National Research Council of the Philippines. Read before the Thirty-sixth Annual Meeting of the Philippine Islands Medical Association, Baguio, May 2-5, 1939. The flowers are usually few from special numerously bracteate branches or stalks; pedicels 3 cm long, fulvous; calyx small, linearly dentate; petals 2 cm long, oblong, glabrate, leathery; torus 5 to 8 mm in diameter. The fruits are many, ellipsoid, averaging 1 cm long, glabrous, upon smooth stalks 2 to 3 times as long.

An endemic tree fairly well distributed from Babuyan Islands and Northern Luzon to Mindanao, in forests at low and medium altitudes.

The bark from younger trees is sometimes used for tying purposes. The same bark, after removing the outer portion, is scraped off into very thin pieces and put into a glass containing a small amount of clean water. The mixture is macerated and then filtered on a piece of clean cloth, and then dropped into sore eyes or inflamed conjunctiva before going to bed and in the morning, every day until the eyes are cured.

### PREPARATION

Extract of the bark of *Phaeanthus ebracteolatus* has been shown to contain a large amount of alkaloids of two types, the tertiary and the quarternary alkaloids. A. C. Santos(1) and J. I. Sulit(3) have each separated a tertiary alkaloid; the former isolated cubical crystals and named them "phæanthine" and the latter, under the supervision of the former, isolated granular crystals to which the name "kalimatine" was given. After removal of these tertiary alkaloids, like the well-known strychnine, atropine, and others, by ether extraction in an alkaline medium, the aqueous portion left still contained a large amount of alkaloid or alkaloids. Alkaloids that could not be extracted by ether in alkaline medium are known as quarternary alkaloids. These quarternary alkaloids of *Phaeanthus ebracteolatus*, which have not yet been isolated in pure form but are present in aqueous or alcoholic solution, are the subject of the present paper.

The fluid extract of *Phaeanthus ebracteolatus* used in the experiments was prepared by percolation of the powdered bark No. 40 of *Phaeanthus ebracteolatus*, 4 parts of alcohol and 1 part of distilled water being used as menstruum. The powder was macerated for 2 days and then percolated at the rate of about 20 drops per minute, and after collecting 850 cc for every kilogram of powder used, the remaining percolate was separated and percolation continued until the percolate became negative to Mayer's precipitation test. The last percolate was then evaporated at low temperature and the residue added to the first collection and made up with menstruum to 1,000 cc for every kilogram of the powder that was employed. The fluid extract thus prepared is of a dark reddish-brown color, with a violet tint on the surface.

A standard quarternary alkaloidal solution or aqueous extract was prepared by taking a certain volume of the fluid extract. evaporating the alcohol, and removing the tertiary alkaloids in alkaline medium by the use of ether or chloroform, and the aqueous layer left, after having been rendered slightly acid to litmus paper, was made up to the original volume of the fluid extract taken. This was considered the standard quarternary alkaloidal solution of Phaeanthus ebracteolatus. Other aqueous extracts, not of standard strength, were also frequently employed. When the aqueous extract of kalimatas was reduced on spontaneous evaporation to semisolid consistency and the semisolid portion weighed and dissolved in water, the soluble or clear portions were designated of 2 per cent strength, if 2 grams of the semisolid extract was used in 100 cc of water. Two and 5 per cent of this type of preparation were employed in the experiments. The quarternary solution, in alkaline, neutral, or acid reaction, reacted with Mayer's reagent by producing precipitation. alkaline solution was somewhat cloudy, dark brown, with a greenish tint on the surface; after acidification the color changed to clear red, or brownish red with a violet tint on the surface.

# PHARMACODYNAMICS

Toxicity.—The pharmacodynamic effects of quartenary alkaloid in aqueous solution and of the fluid extract of Phaeanthus ebracteolatus have been found practically identical, so that a pharmacodynamic study of one preparation will represent the activity of the other. The minimum toxic doses were determined in different animals and in frogs representing cold-blooded animals: the minimum fatal dose was 0.5 cc of the fluid extract or of the standard quarternary alkaloidal solution per kilogram body weight, while in mammals, as represented by mice, cats, and dogs, the subcutaneous or intramuscular minimum fatal dose was 1 cc per kilogram body weight. The prominent symptoms with fatal doses were depression, as shown by sluggish movement, and inability to support the body, and then by preliminary increased respiration followed by decreased and difficult respiration until respiration ceased. In a similar way the heart beat faster and then slowed and stopped in all cases a few minutes after cessation of the respiration. An example of the effects on a dog of a maximum nonfatal dose of the extract of kalimatas, or Phaeanthus ebracteolatus, is shown in Table 1.

As shown in Table 1 the heart rate and respiratory rate were affected, being first increased and then decreased to a

Table 1.—Effects on a dog of a maximum nonfatal dose of kalimatas extract.

Time.	Pulse rate per minute.	Res- piratory rate per minute.	· Remarks.
9:55 9:59	100	22	Observation taken two times.  Injection into the gluteral region of 5 cc standard aqueous extract of kalimatas. Pain shown during injection.
10:09	116	36	Rubbing with mouth the place of injection.
10:20	. 124	- 28	Trembling of the legs.
10:30	142	26	Trembling of body and legs.
10:40	138	26	Lying on his abdomen. Would not stand on light stimulation.
10:50	136	26	Head drooping. Unwilling to stand.
11:00	134	24	Lying on the left side of hind body and on chest.
11:10	184	24	Lying on the left side of body and on chest. Pupils dilated.
11:20	128	20	Do.
11:30	134	16	Do.
11:40	134	18	Stood but soon laid down again.
11:50	136	16	Could not stand even after strong stimulation.
12:00	a 64	12	Expiration irregular and difficult. Much salivation.
12:10	46	12	Do.
12:20	a 68	14	Do.
12:40	104	18	Expiration slightly difficult and helped by jerky movements. Much salivation.
12:50	100	24	Regular respiration.
1:00	100	24	Do.
March 8			Strong, like normal.
March 9			Do.
March 10			Do.

a Irregular.

critical period when the dog seemed about to die, approximately about 2½ hours after administration of the drug. The sudden change of the pulse rate occurred 2 hours after injection, when it decreased from 136 to 64 and then irregularly to 46, while respiration was difficult and expiration accompanied by jerky movements. Much saliva flowed from the mouth, and for about 40 minutes the dog was in serious condition, lying helpless, with jerky expiration; but soon thereafter the pulse improved and the respiration also increased, and 50 minutes after the onset of the critical condition the pulse rate and respiration became normal, the dog stirred and stood, weakly, especially on the hind legs. Thereafter he acted normal and continued to act normal during the 3 days of subsequent observation.

When the minimum fatal dose of 1 cc standard extract of kalimatas per kilogram body weight was injected in a 7-kilogram female dog, 7 cc in all being injected intramuscularly, the

animal died 28 minutes after injection, with cessation first of respiration and then of the heart. On autopsy the findings were: (a) collapsed lungs; (b) distended vena cava and right heart; (c) distended blood vessels of the mesentery, and (d) congestion of the liver.

Effect on blood pressure.—The most important effect of quarternary alkaloidal solution of Phaeanthus ebracteolatus or its fluid extract, observed on intravenous injection, was a fall in blood pressure. A distinct fall was produced with 0.1 cc and was correspondingly increased with increasing doses as indicated in tracings 1 to 5 (Plate 2). When the first dose was a large one, like 0.5 cc, and after recovery another injection was given of a diminishing dose, the subsequent diminishing doses produced a corresponding diminution in the blood-pressure falls. The same dose under similar conditions in the same animal produced practically identical falls of blood pressure, and this similarity in effects by equal doses was used as the basis for the employment of the blood-pressure method of assaving any of the alcoholic or aqueous preparations of the quarternary base of Phaeanthus ebracteolatus. In a series of animals the falls produced by the same dose of quarternary alkaloidal solution were different, more generally in smaller animals and where the initial blood pressure before injection was high. The recovery to normal blood pressure after each injection was slow and gradual, and the recovery was more gradual as the dose was increased. When a very large dose was administered, such as 1 cc of the fluid extract of the standard quarternary alkaloidal solution, the fall in blood pressure produced was so great as to reach nearly the base line. In tracing 6 (Plate 3) the systolic fall was from 142 mm Hg to 14 mm Hg. The respiration in tracing 7 (Plate 4) first accelerated and increased in amplitude, then decreased and finally stopped, and in tracing 6 a deep respiration was observed followed by irregularity and cessation, with the heart still beating for sometime. The blood pressure after cessation of respiration rose for a short time to fall again, or continued to fall until the heart ceased to beat, as shown in tracings 6 and 7. When artificial respiration was given, after cessations of respiration and contraction of the heart, recovery was observed to be difficult. However, when artificial respiration was given soon after respiration ceased, with the heart still beating, the heart continued to heat, maintaining the low-blood pressure for some time and gradually recovering, in one hour or more, with the eventual restoration of respiration as shown in tracing 7.

The heart rate during the fall after intravenous injection of quarternary alkaloidal solution of *Phaeanthus ebracteolatus* either increased slightly or decreased after small doses and nearly always slightly decreased after large doses. The decrease was observed after small doses where the heart rate was fast previous to the injection and either distinctly increased or slightly decreased when the heart rate was slow previous to the injection. The heart contraction in intact animals during the fall after intravenous injection of quarternary alkaloidal solution of *Phaeanthus ebracteolatus* was greatly increased, as shown in the cardioplethysmograph tracing 8 (Plate 5).

The intestinal volume during the fall first increased, and then decreased while the blood pressure was returning to normal. On the other hand, the kidney volume seemed to follow the blood-pressure effects, first falling and then rising, with definite relation to the fall and then gradual rise of the blood pressure after injection of the quarternary alkaloidal solution of *Phaeanthus ebracteolatus*. The preceding effects were generally obtained in dogs with low blood pressure due to the use of morphine as general anæsthetic. However, when the blood pressure was previously raised by injecting curare to paralyze the muscles and then a toxic or convulsive dose of strychnine, the effects of the aqueous extract of kalimatas on intestinal and kidney volumes were greatly increased as shown in tracing 9 (Plate 6).

The fall of blood pressure produced by quarternary alkaloidal solution of *Phaeanthus ebracteolatus* on a dog with cut vagus nerves was almost as much as with intact vagus nerves. The fall was not prevented by previous administration of atropine.

Comparison with nitroglycerin and acetylcholine.—With a 2 per cent solution of acetylcholine chlorate and beginning with a small dose, like 0.05 cc, injected intravenously, the fall of blood pressure produced was sudden, as shown in tracing 10 (Plate 7). If after administration of atropine the same dose was repeated or twice as large a dose given, as shown in the tracing, the typical fall was not obtained, but instead the blood pressure rose and remained high for some time. On the other hand, intravenous injection of the quarternary alkaloidal solution of Phaeanthus ebracteolatus, after application of atropine, produced the characteristic fall of blood pressure shown in the same tracing 10. Repetition of injection of acetylcholine and quarter-

71. 4

nary solution, with the dog still under the influence of atropine, produced similar effects, rise with acetylcholine and fall with quarternary alkaloids of *Phaeanthus ebracteolatus*.

The fall in blood pressure produced by quarternary alkaloidal solution of Phaeanthus ebracteolatus was in some points different from that produced by nitroglycerin. Tracings 11 and 12 (Plate 8) show the effects of nitroglycerin and of the quarternary alkaloidal solution, respectively. With doses which produced an almost identical fall in blood pressure or even a greater fall in the case of nitroglycerin, the recovery was gradual in the case of the quarternary alkaloidal solution and rather rapid after nitroglycerin. This difference has been observed to remain constant in several experiments. Other differences are in the heart rate during the fall and in the effects on the intestinal volume. While the heart rate during the blood-pressure fall after nitroglycerin was greatly increased in the case of quarternary alkaloidal solution, the majority of the cases showed slight slowing down, no change, or a slight increase; but the increase, if there was any, was not as marked as in the case of nitroglycerin. The effects on the intestinal volume showed greater increase or rise in the record after injection of nitroglycerin than that produced by injection of kalimatas.

# OTHER PHARMACODYNAMIC EFFECTS

In a perfusion experiment on a separated leg of a dog, where the flow was made slow by either injection of epinephrine or pitressin, injection of quarternary alakaloidal solution of *Phaean-thus ebracteolatus* caused a sudden increase in the flow.

An excised heart of frog, prepared by Straub-Fuehner's(2) method, showed, after addition of a trace of the quarternary alkaloidal solution of *Phaeanthus ebracteolatus*, a slight increase in the amplitude of contraction as shown in tracing 13 (Plate 9). In tracings 14 and 15 of the same plate the response to  $\frac{1}{2}$  drop of the quarternary alakaloidal solution was always decreased in the amplitude of contraction. When 1 drop was employed, the heart contraction diminished and ceased after some time, but was again revived by a change of the Ringer solution, as showing in tracing 6.

The natural movement of an excised intestine from a cat suspended in aërated Locke-Ringer solution, and kept warm at 37° to 38°C., was decreased or stopped by addition of quarternary alkaloidal solution as shown in tracing 17 (Plate 10).

In the tracing the intestinal contraction was resumed immediately after addition of pilocarpine. In a similar way the movement of the excised intestine, augmented by pilocarpine, was stopped by addition of quarternary alkaloidal solution as shown in tracing 18 (Plate 10). In tracing 19 (Plate 10) the intestinal contraction was diminished by addition of aqueous extract of kalimatas and stopped by further addition of another dose, made to contract by addition of barium chloride, and again relaxed by addition of a large dose of kalimatas solution. It will be noticed in the experiments that relatively large doses were necessary to produce a decided effect of depression on the intestinal movement, especially when this was previously stimulated by pilocarpine or barium.

In a similar way, experiments were carried out by suspending an excised tracheal muscle from a dog in Locke-Ringer solution. aërated and kept at a temperature of 37° to 38°C. The results are shown in tracings 20 and 21 (Plate 11). The tracheal muscle was made to contract by addition of pilocarpine in one and barium in the other. Tracing 20 shows the stimulant effect of pilocarpine hydrochloride on an excised tracheal muscle, counteracted by aqueous extract of Phaeanthus ebracteolatus. Ordinarily, without addition of another drug, after reaching the peak of maximum contraction produced by pilocarpine, the muscle began to relax gradually and then remained contracted to the same degree or was even contracted more, so that the record will show an abrupt rise followed by a gradual fall to a uniform level, and another slight rise after about 20 to 30 minutes. fall never reached one half but generally reached a third or less of the maximum height of contraction. In the experiment the addition of 2 cc of standard quartenary alkaloidal solution of Phaeanthus ebracteolatus caused a distinct fall in the record or a relaxation of the muscle, but the depressant action was not sufficient to produce a complete relaxation. In a similar way the effect of depression, as shown in tracing 21, with 2 cc of standard quarternary alkaloidal solution on excised tracheal muscle made to contract by barium chloride, was not sufficient to relax the muscle completely.

The effect of quaternary alkaloidal solution on the contraction of an excised uterus from a cat suspended in Locke-Ringer solution, aërated and kept at a temperature of 37° to 38°C., is shown in tracings 22 and 23 (Plate 12). In both tracings the uterine contraction was augmented by addition of pitocin in one and his-

tamine in the other. During the height of contraction solution of quarternary alkaloids of *Phaeanthus ebracteolatus* was added in each case, and distinct relaxation was obtained in both.

Application of one or two drops of quarternary alkaloidal solution on the eye of a cat or a dog produced slight reddening of the conjunctiva with no change in the size of the pupil. The reddening disappeared in about 12 minutes after application of one drop and in about 20 minutes after 2 drops.

### DISCUSSION

The fall of blood pressure produced by any drug is occasioned either by dilatation of the blood vessels, especially of the splanchnic region, or by decreased output of the heart due to vagus stimulation or direct depression of the heart itself, or by both. Vagus stimulation was not concerned in the fall of blood pressure produced by quarternary alkaloidal solution of Phaeanthus ebracteolatus, for the same effect was produced after cutting the vagus nerves or after paralysis of the vagus receptive mechanism by atropine. Direct depression of the heart alone could not explain the falls in blood pressure, for with small doses the heart contraction was even increased in rate and amplitude. while with large doses slight slowing and distinct diminution of the heart contraction were produced. In view of this, the fall in blood pressure produced by small doses of quarternary alkaloidal solution could be explained by dilatation of the blood vessels, while after moderate and large doses the fall was brought about by dilatation of the blood vessels and depression of the heart. The dilatation of the blood vessels was confirmed by increase in the organ volume of the intestine during the fall of blood pressure and increase in the amount of flow in the perfusion experiment. The action of a drug in dilating the blood vessels may be due to either direct depression of the muscles, to a certain action of the drug on the nerves, or to a combination of the two; but since the quarternary alkaloidal solution relaxed all smooth muscles, irrespective of their innervation. it may be concluded that the dilating effect of quarternary alkaloidal solution of Phaeanthus ebracteolatus was due to depression of the smooth muscles of the blood vessels.

### CONCLUSIONS

1. The quarternary alkaloid in aqueous or alcoholic solution of *Phaeanthus ebracteolatus* depresses the smooth muscles, espe-

cially of the blood vessels, and as a result the blood pressure falls. With moderate and large doses the fall in blood pressure produced by quarternary alkaloids of *Phaeanthus ebracteolatus* may be partly due also to depression of the heart.

- 2. With sufficient large doses it also depresses the intestine, tracheal muscles, and uterus, and is able to counteract the stimulant effects produced by pilocarpine and barium on the intestine and tracheal muscles, and by pitocin and histamine on the excised uterus.
- 3. The fall in blood pressure produced by quarternary alkaloids of kalimatas was of longer duration than that produced by nitroglycerin and acetylcholine, two well-known drugs that lower the blood pressure.
- 4. With fatal doses the respiration was first increased temporarily then decreased and stopped. The blood pressure fell to as low as 14 mm or less of Hg and kept at that level until it was raised by asphyxia, and then dropped to zero level through cessation of the heart beat a few minutes after the failure of respiration. When artificial respiration was applied after cessation of respiration, the heart continued to beat, maintaining the low blood pressure for some time, and then gradually recovered to normal inside of 1 hour or more after administration of the drug.

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# ILLUSTRATIONS

[Illustrations prepared for publication by Francisco Rafael.]

### PLATE 1

Close view of trunk of Phaeanthus ebracteolatus.

### PLATE 2

Tracings 1 to 5 of blood-pressure falls after intravenous injection of fluid extract of *Phaeanthus ebracteolatus* on a 10.5-kilogram female dog under morphine, 15 mg per kilogram body weight, and ether during operation. Tracing 1, 0.1 cc injection; 2, 0.2 cc injection; 3, 0.3 cc injection; 4, 0.4 cc injection; 5, 0.5 cc injection. Intervals on scale are equal to 6 seconds.

# PLATE 3

Tracing 6, respiration and carotid blood pressure of a 7.4-kilogram female dog, under morphine sulfate, 15 mg per kilogram body weight, and ether during operation. Intervals on scale are equal to 6 seconds.

### PLATE 4

Tracing 7, respiration and carotid blood pressure after intravenous injection of 1 cc of quarternary alkaloids (1:1 solution) of *Phaeanthus ebracteolatus*. Intervals on scale are equal to 6 seconds.

### PLATE 5

Tracing 8, heart contraction and blood pressure. Intervals on scale are equal to 6 seconds.

### PLATE 6

Tracing 9, intestinal volume, blood pressure, and kidney volume of a 10.3-kilogram female dog under morphine sulfate curare, and strychnine sulfate.

#### PLATE 7

Tracing 10, quarternary solution of *Phaeanthus ebracteolatus* compared with acetylcholine. Intervals on the scale are equal to 6 seconds.

### PLATE 8

Tracings 11 and 12, blood pressure falls produced by quarternary solution of *Phaeanthus ebracteolatus* and by nitroglycerin. Intervals on scale are equal to 6 seconds.

### PLATE 9

Tracings 13 to 16, effect of quarternary alkaloidal solution on contractions of an excised frog's heart.

# PLATE 10

Tracings 17 to 19, effect of addition of kalimatas extract to excised cat's intestine in Locke-Ringer solution. Intervals on scale are equal to 6 seconds.

# PLATE 11

Tracings 20 and 21, effect of pilocarpine HCl, barium chloride, and standard quarternary alkaloidal solution of *Phaeanthus ebracteolatus* on an excised tracheal muscle in 200 cc Locke-Ringer solution. Intervals on scale are equal to 6 seconds.

# PLATE 12

Tracings 22 and 23, effect of pitocin, kalimatas extract, adrenaline, pitressin, and histamine on an excised cat's uterus (nonpregnant) in 200 cc Locke-Ringer solution. Intervals on scale are equal to 5 seconds.



PLATE 1.









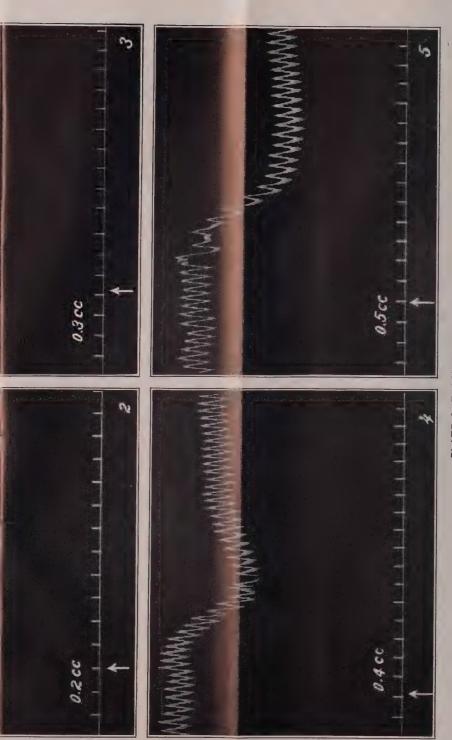
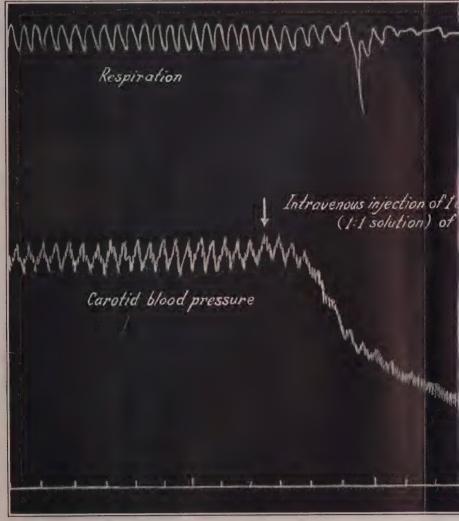
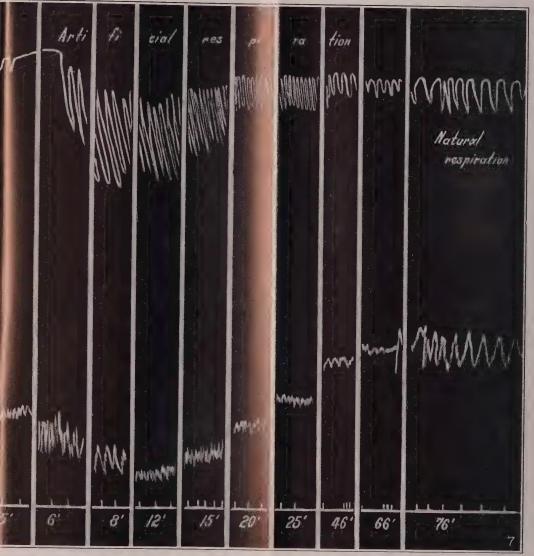
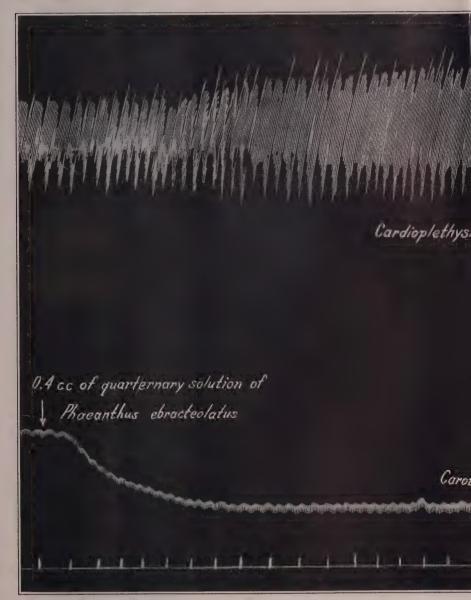


PLATE 2, TRACINGS 1 TO 5.





RACING 7.





TE 5. TRACING 8.

Intestinal volume Blood pressure Under curare, artificial respiration, and 3ce strychnine SU4, 01% (toxic dose) Kidney volume Intravenous injection of 0.2cc quarternary aqueous extract of kalimatas, 4.02 p.m.



TRACING 9.

GARCIA: PHARMACOLOGY OF PHAEANTHUS EBRACTEOLATUS.]

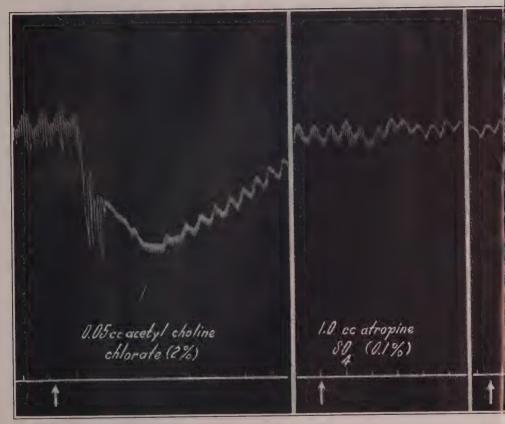
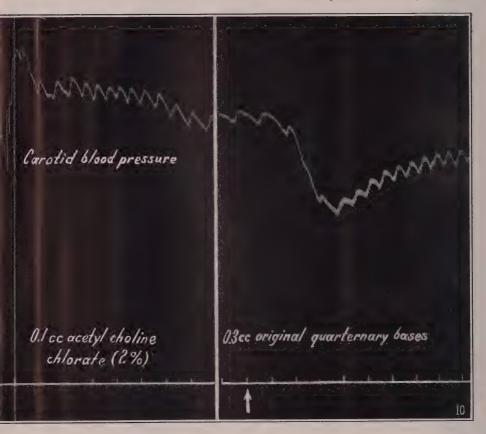


PLATE 7. T



ACING 10.

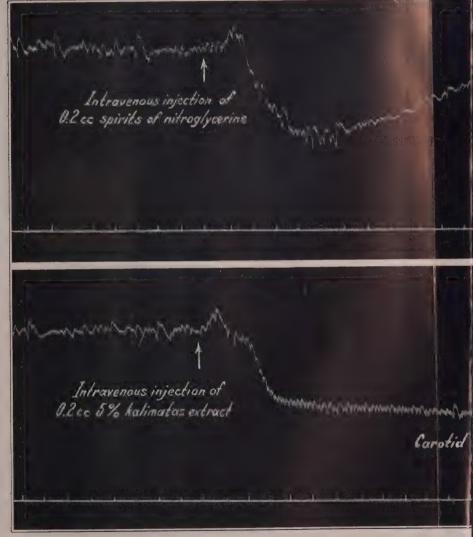
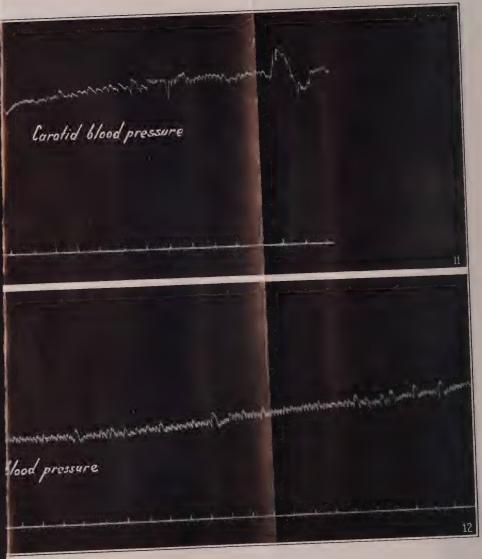
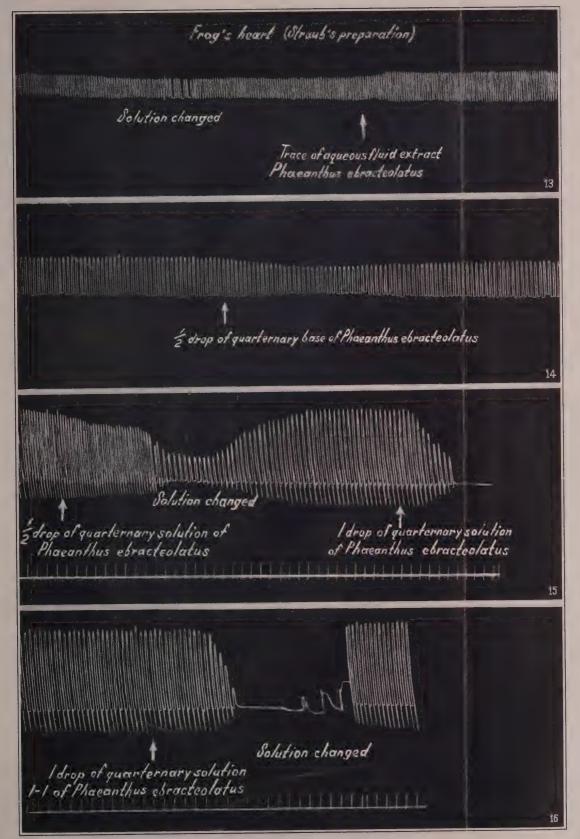
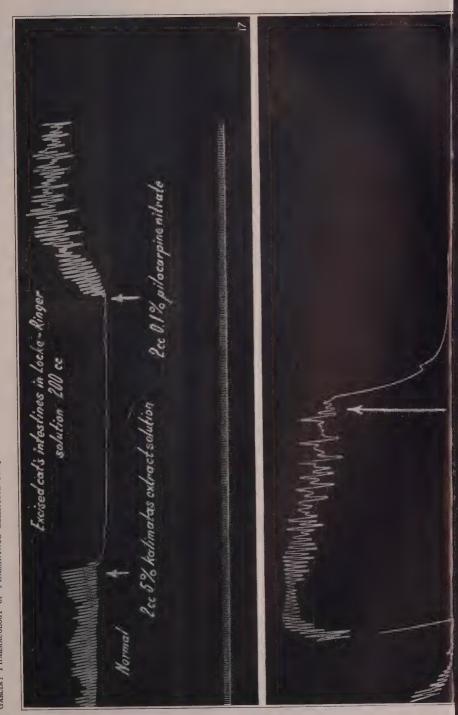


PLATE 8. TE









10 ce 5% kalimatas
extract

t
2cc 10% Ball,

2005 % Kalimatas extract

PLATE 10. TRACINGS 17 TO 19.

Lec slandard quarternary alkaloidal solution of Phaeonthus ebracteolatus

Excised tracheal muscle from dog in 200 cc Locke - Ringer's solution

0.3 cc 1% pilocarpine HCL

Zee standard quarternary alkaloidal solution of phaeanthus ebracteolatus

lec 10% barium chloride



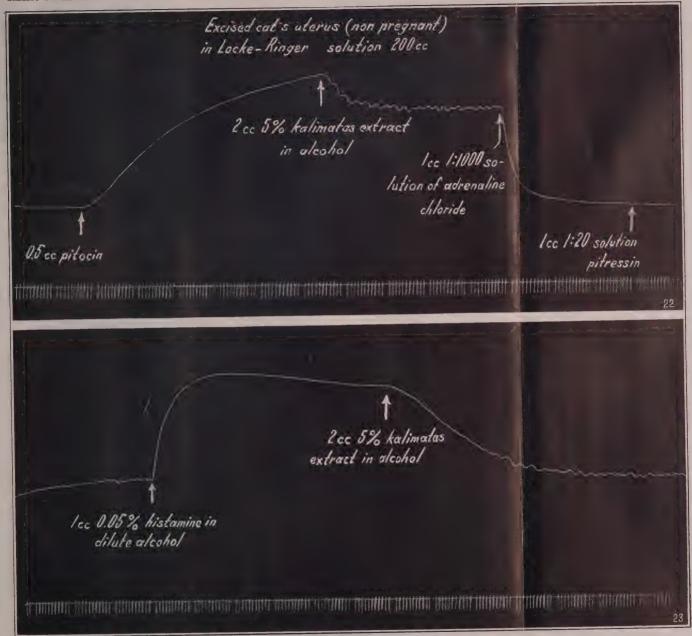


PLATE 12. TRACINGS 22 AND 23.



### TWO NEW PHILIPPINE GOBIOIDS

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### TWO PLATES

In my recent collection of fish in Dagupan, Pangasinan Province, I have had occasion to examine two gobioid specimens of the genera *Apocryptodon* and *Lophogobius*, which are apparently new. The original descriptions of these two genera are included in this short paper, with a discussion of the relationship of the genus *Lophogobius* to the genus *Cristatogobius*.(1)

### Genus APOCRYPTODON Bleeker

Apocryptodon BLEEKER, Esquisse Arch. Neerl. sc. ex. et nat. 9 (1874) 827 (Apocryptes madurensis Blkr.).

Body very elongate, anteriorly subcylindrical, posteriorly compressed, covered with 40 to 60 deciduous cycloid scales. Head posteriorly subcylindrical, anteriorly a little depressed, scaled above behind eyes, laterally scaled from under eye on preoperculum and operculum. Eves in anterior half of head, bony interorbital less than 1 diameter of eye. Snout a little more than 1 diameter of eye. Nostrils not tubular. Mouth nearly horizontal, jaws subequal. Teeth in both jaws in one row, in upper jaw caninoid, in lower jaw horizontal, truncate to bilobate, after symphysis on each side a canine. The upper jaw has a curvity, receiving the tip of the lower jaw. Tongue rounded, nearly totally adnate to the floor of the mouth. Gill openings about as long as breadth of base of pectoral fin, isthmus broad. Inner edge of shoulder girdle without fleshy flaps. Dorsal fins close together, first dorsal with 6 spines; second dorsal with 23 to 24 rays; anal with 22 or 23 rays; ventrals united, oblong under pectorals. Pectorals without free rays, base scaled; caudal pointed.

APOCRYPTODON LOMBOYI sp. nov. Plate 1.

First dorsal VI; second dorsal I, 20 or 21; anal I, 20 or 21. Head low, elongate, little broader than body, 4 to 4.4 times in length and slightly greater than caudal; snout slightly convex, with a fleshy fold above upper jaw, 5 to 6 times from tip of snout to origin of first dorsal; mouth large and oblique, lower jaw a little less than upper jaw; posterior angle of maxillary extending beyond posterior margin of eye; eyes small, dorso-lateral, upward gazing, close together, equal to orbital space; a median terminal hump on snout before eyes; upper jaw with 16 to 22 teeth, anterior teeth generally long and curving inwardly over lower jaw; 18 to 26 bifid teeth outwardly inclined and not extending as far back as teeth of lower jaw; few round and small dusky spots on side of head from gill opening to an angle below eye; scales on opercle small and bluish, concolorous to belly.

Body subcylindrical, low and elongate, very little elevated and almost parallel to ventral profile; 8 or 9 dusky blotches saddle dorsal side from head to caudal; another series of 5 or 6 blotches in middle portion of body; 58 to 60 scales in longitudinal series, 15 to 18 in transverse series, and 27 to 30 before first dorsal.

Pectoral fins broad and pointed, 2.3 to 3 times in base of anal, longest rays extending back at an angle below base of fifth spine or first blotch in the side; base of pectoral naked, mottled with fine round specks, lower edge of pectoral fin dusky with a narrow grayish outline; first dorsal higher than second, last or sixth spine, placed very far from fifth, scarcely reaching origin of second dorsal when depressed; second dorsal and anal alike in shape, low, reaching base of elongate, pointed caudal when depressed; ventrals either oblong or elongate, under pectoral; color not much changed in spirit after several days, grayish and freckled with blackish specks or dots all over body and head; scales easily dislodged in living specimens.

The present new species shows overlapping characters with the other species so far known locally. It is very close to A. sealei and A. montalbani in having blotches in the middle of the side of the body, but very distinct from anyone of them in having prominent blotches saddling the dorsal.

Table 1 shows comparison of the dentition, scale count, fin rays, and number of specimens on which the original description is based, for every Philippine species of the genus.

Here described from 27 specimens, the type, No. 31128, and 26 cotypes, 50 to 65 millimeters long, obtained from fish vendors in Dagupan, Pangasinan Province, Luzon. The specimens are kept in the ichthyological collection of the Division of Fisheries, Department of Agriculture and Commerce, Manila.

<sup>&</sup>lt;sup>1</sup> Formerly the Fish and Game Administration, Bureau of Science, Manila.

TABLE 1 Dentition,	scale o	count,	and	fin	rays	of	Philippine	species
of Apocryptodon.								

A	Spe-	Dentition.		S	cale coun	Fin rays.		
A poery ptodon.	cimens.	Upper.	Lower.	Long.	Trans.	Predors.	Dorsal.	Anal.
lomboyi	27	16-22	18-26	58-60	15-18	27-80	VI, I,20 I,21	I,20 I,21
montalbani	1	24	24	56	16	24	VI, I,21	I,21
sealei	1	20	28	52	18	22	VI, I,21	I,22
taylori	1	18-20	16	60		84	VI, I,22	I,21

This species is named for Julian S. Lomboy, an artist, for his patience in sketching this fish.

# DISCUSSION OF THE GENERA LOPHOGOBIUS GILL AND CRISTATOGOBIUS HERRE

The genus Lophogobius (Gill, 1862), which was established from the type, Gobius crista-galli Cuv. & Val., is represented by species inhabiting the West Indies and Florida.(1) The genus Cristatogobius Herre was established from 3 specimens of the type C. lophius. Koumans,(2) in his preliminary revision of the genera of gobioid fishes with united ventral fins, claims that Cristatogobius Herre is closely allied to Lophogobius Gill, confirming a similar remark of Herre in the original description of Cristatogobius.

In the original description of *Cristatogobius*, Herre mentions a single row of teeth in *Lophogobius*, a character of dental variation that warrants establishing *Cristatogobius* as a new and separate genus, having 4 rows of teeth. Koumans saw in the specimens of *Gobius crista-galli*, the type of *Lophogobius*, that the teeth were placed in several rows, the outer row being enlarged, and lacked canines. Thus, while *Cristatogobius* and *Lophogobius* have teeth in rows, *Cristatogobius* differs from *Lophogobius* in having a pointed caudal and canines.

#### Genus LOPHOGOBIUS Gill

Lophogobius GILL, Proc. Acad. Nat. Sci. Phila. 14 (1862) 240 (Gobius crista-galli Cuv. & Val.).

Body elongate, compressed, covered with 25 to 30 scales, ctenoid on most parts of body, becoming more cycloid on nape, breast, and belly. Head compressed, with swollen cheeks, naked. Nape with fleshy, naked crest, scales on nape beginning from a vertical from posterior margin of preoperculum, eyes in ante-

rior half of head, bony interorbital and snout about 1 diameter of eye. Snout blunt. Anterior nostril in a short tube. Mouth oblique, lips thick, jaws subequal. Teeth in both jaws in several rows, outer row a little enlarged, canines lacking. Tongue rounded. Some mucous canals diverging under eye and some short canals around eye, some on operculum, a longitudinal one on preoperculum, and one along lower jaw to posterior margin of preoperculum. Open pores, one median of posterior nostril, one in interorbital space close before beginning of nuchal crest, one after each eye, some along upper and posterior margins of preoperculum. Gill openings as long as breadth of base of pectoral fin, isthmus broad. Inner edge of shoulder girdle without fleshy flaps. Dorsal fins separate, first dorsal with 6 spines, second dorsal and anal with 7 to 10 rays, ventrals united, oblong, under pectorals. These without free silklike rays; caudal rounded.

### LOPHOGOBIUS NONATOÆ sp. nov. Plate 2.

First dorsal VI; second dorsal I, 9, rarely I, 10; anal I, 9.

Head a little deeper than body, 3.1 to 4 times in length, dorsal profile above eyes forming a curve to origin of dorsal, snout short and slightly convex, equal to eye, eyes forming a linear interorbital space; posteriad of eyes on nape a skinny crest or comb extending back to first dorsal, and with a base equal to or less than body in depth; mouth almost vertical and with a projecting chin; posterior angle of maxillary in line with anterior of eye or a little over; nape with small scales; rows of mucous canals on head; two behind eyes on nape, one double horseshoeor S-curved below eye on preopercle, some on operculum, around eye, one on lower edge of chin; teeth in several rows in each jaw, those of outer and inner rows enlarged, posterior lateral pair of large backward-curved canines in outer row of lower jaw, tongue rounded at tip.

Body compressed laterally, scales larger posteriorly from first dorsal to caudal, 31 to 33 in longitudinal series, 11 in transverse series; all fins except pectoral dusky, in life grayish with green specks in yellowish shade on cheeks, becoming prominent on sides above pectorals, assuming rod-shaped blotches; reddish streaks on base of pectoral, anteriad of caudal a number of round specks of similar tinge as that

above pectorals; spinous dorsal with flexible spines, third and fourth longest, longest spine in line with upper margin of base of pectoral, its base 2 times longest spine; second dorsal and anal similar in shape, anal a little lower in height, their longest rays reaching caudal when depressed; in life second dorsal cross-barred with reddish bars in four rows; first dorsal with greenish specks; pectoral long and pointed, its long rays extending at a point below second ray of second dorsal, 2 to 2.8 times body; caudal pointed and longer than head; ventrals originating below pectorals and extending as far as anal papillæ; in alcohol or spirit slatey blue, specks on body becoming white dots.

The present species is very close to *Cristatogobius lophius* and *Gobius crista-galli* in having teeth in several rows, and in the presence of mucous canals and pores on the head. It resembles *C. lophius* and differs from *Gobius crista-galli* in having a pointed caudal. It is very distinct from *C. lophius* in the absence of crossbands on the sides, in having fewer rays in the second dorsal and anal, and in the greater number of scales in longitudinal and transverse series.

Here described from type specimen No. 31129 and 49 cotypes, 18 to 49 millimeters long, collected by Miss Susana G. Nonato from fishponds in Dagupan, Pangasinan Province, Luzon, and kept in the ichthyological collection of the Division of Fisheries, Department of Agriculture and Commerce, Manila.

Nonatoæ after the collector.

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### ILLUSTRATIONS

 $\begin{array}{c} {\rm PLATE} \;\; 1 \\ {\it A pocryptodon} \;\; lomboyi \;\; {\rm sp.} \;\; {\rm nov.} \end{array}$ 

PLATE 2
Lophogobius nonatoæ sp. nov.



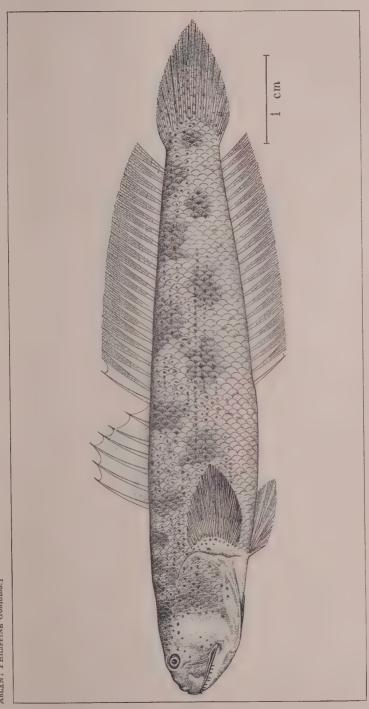


PLATE 1. APOCRYPTODON LOMBOY! SP. NOV.



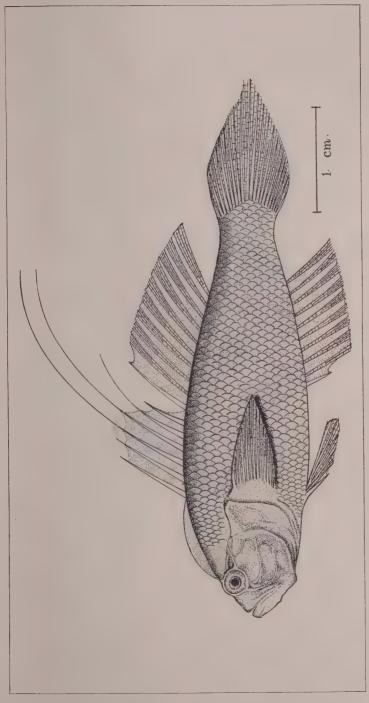
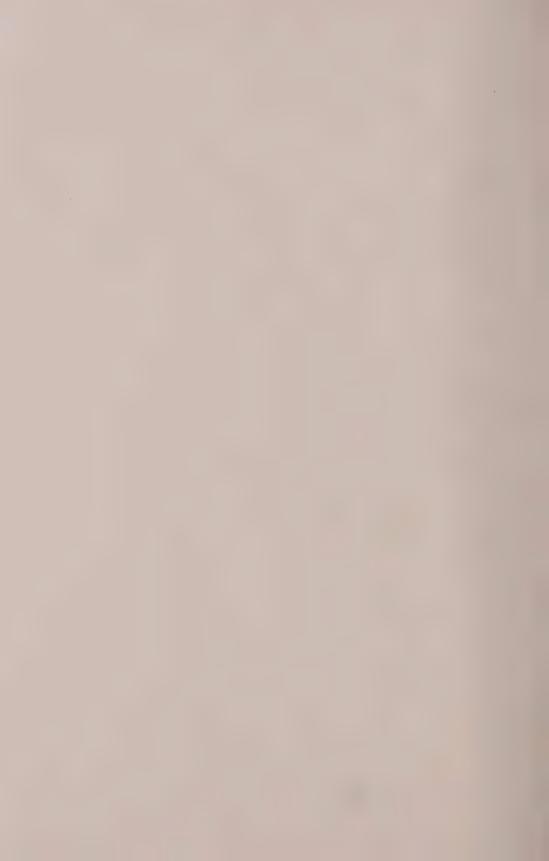


PLATE 2. LOPHOGOBIUS NONATOÆ SP. NOV.



## HELMINTH PARASITES OF THE SNAKES OF BURMA, I

### TREMATODA

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FIVE TEXT FIGURES

Investigation of the helminth fauna of the snakes of Burma during the period of the author's connection with the Helminthological Institute of the University of Rangoon, from June, 1930 to December, 1938, has yielded a large number of helminths from various locations in the host. Trematodes have been obtained from the intestine, gall bladder, and bile ducts; adult cestodes from the intestine; free larval forms from the body wall and mesentery; and encysted larvæ from the intestinal wall. Nematodes were obtained from the esophagus, stomach, intestine, and rectum. Acanthocephala were found only rarely, and invariably as cysts on the intestinal wall, and pentastomids in the lungs. Examination of the heart, liver, kidney, ureter, and oviduct were without result. A total of 212 snakes were examined during this period. The snakes, except the pythons, were obtained either from Rangoon and suburbs or from Thandaung, a town in the Shan plateau, situated at an elevation of approximately 4.500 feet above sea level. The snakes from Rangoon and suburbs caught alive or dead were dissected either by Professor Meggitt or by his junior staff, and those from Thandaung have been dissected exclusively by Professor Meggitt. The author is extremely grateful to Professor Meggitt for the facilities afforded him for study and identification of some of these snakes, and the collection of parasites from them. Fortyone species of snakes belonging to different families have been dissected: Table 1 gives a list of the species dissected and the number found infected.

The trematode parasites of snakes from Burma have already been described by Bhalerao and Gogate in various publications; the present report is intended to throw light on important controversial points recently raised by workers in India and abroad, in connection with the structure and classification of these worms. An account of two new species of parasites is also included. The author wishes to express his gratitude to Dr. G. D. Bhalerao for the loan of the type slide of *Styphlodora nicolli*, which has facilitated the study of the new species of *Styphlodora* here described.

TABLE 1 .- Species of snakes dissected and number found infected.

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Host.	Number dissected.	Number infected.
Amblycephalus carinatus (Boie, 1828)	1	1
Boiga cyanea (Dumeril and Bibron, 1854)	2	2
Boiga multimaculata (Boie, 1827)	6	3
Bungarus candidus (Linnæus, 1758)	2	2
Bungarus candidus multicinctus (Blyth, 1861)	1	1
Bungarus fasciatus (Schneider, 1801)	3	2
Cerberus rynchops (Schneider, 1799)	4	0
Chrysopelea ornata (Shaw, 1802)	6	5
Dryocalamus davisonii (Blanford, 1878)	2	0
Elaphe radiata (Schlegel, 1837)	4	4
Enhydris enhydris (Schneider, 1799)	25	22
Homalopsis buccata (Linnæus, 1754)	2	2
Lycodon aulicus (Linnæus, 1758)	27	17 *
Lycodon jara (Shaw, 1802)	1	1
Lycodon travancoricus (Beddome, 1870)	1	1
Naja hannah (Cantor, 1836)	1	1
Naja naja (Linnæus, 1758)	4	2
Natrix chrysarga (Schlegel, 1837)	1	1
Natrix himalayanus (Günther, 1858)	1	0
Natrix parallelus (Anderson, 1879)	9	9
Natrix piscator (Schneider, 1799)	17	14
Natrix punctulata (Günther, 1858)	1	1
Natrix stolata (Linnæus, 1758)	10	7
Oligodon albocincta (Cantor, 1839)	1	1
Oligodon arnensis (Shaw, 1802)	1	1
Oligodon cruentatus (Günther, 1868)	4	4
Oligodon cyclurus (Cantor, 1839)	15	7
Oligodon violaceus (Cantor, 1839)	16	4
Passerita prasina (Boie, 1827)	1	1
Psammodynastes pulverulentus (Boie, 1827)	1	1
Ptyas korros (Schlegel, 1837)	8	8
Ptyas mucosus (Linnæus, 1758)	15	14
Python molurus (Linnæus, 1758)	1	1
Python reticulatus (Schneider, 1801)	2	2
Sibynophis collaris (Boulenger, 1893)	1	1
Trimeresurus gramineus (Shaw, 1802)	8	8
Trimeresurus monticola (Gray, 1853)	1	1
Typhlops diardi (Schlegel, 1837)	3	0
Vipera russelli (Shaw, 1797)	2	2
Xenelaphis hexagonatus (Blyth, 1856)	1	0
Xenopeltis unicolor (Reinwardt, 1827)	3	3

Only cysts were found.

### Family LEPODERMATIDÆ Odhner, 1911

Subfamily STYPHLOTREMINÆ Beer, 1924

Genus STYPHLODORA Looss, 1899

### STYPHLODORA DENTIPHARYNGEATA sp. nov. Text fig. 1.

Three specimens were obtained from a single host. Body moderately long, 3.15 to 3.3 mm, thin, cylindrical, maximum

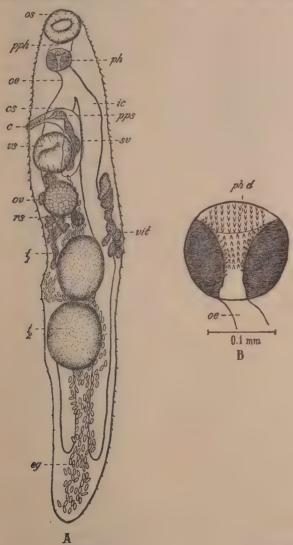


Fig. 1. Styphlodora dentipharyngeata sp. nov. A, entire; B, pharynx, enlarged.

breadth 0.5 to 0.57 mm. Cuticle with stout spines extending throughout entire length of body and arranged in transverse rows, spines in anterior part of body more condensed than in posterior. Oral sucker terminal, subspherical, 0.19 to 0.2 by 0.215 to 0.25 mm. Ventral sucker subspherical, 0.23 to 0.265 by 0.2 to 0.26 mm, at 0.66 to 0.74 mm from anterior end of body, provided with spines. Genital pore median, a little anterior to ventral sucker. Prepharynx approximately 0.03 mm long. Pharynx large, subglobular, 0.1 to 0.14 by 0.12 to 0.15 mm. bearing strong dentitions along its inner margins and leading into a short and wide esophagus 0.1 to 0.12 mm long. tinal cæca ending 0.42 to 0.54 mm from posterior end. large, oval, 0.3 to 0.41 by 0.22 to 0.28 mm and 0.33 to 0.415 by 0.234 to 0.332 mm, respectively, postovarian, entire, oblique, lying one immediately anterior to the other, usually not overlapping. Vas deferens along each side of body, meeting immediately anterior to ovary and posterior to ventral sucker to form a common duct. Cirrus sac 0.35 to 0.43 mm long, crescentshaped, on dorsal side of ventral sucker, reaching almost to anterior level of ovary. Vesicula seminalis curved, occupying most of space in cirrus sac, followed by a narrow pars prostatica. Cirrus muscular, spiny, often everted. Ovary spherical, 0.2 to 0.22 mm in diameter, lying slightly to right of median line, a little posterior to ventral sucker. Receptaculum seminis pearshaped, 0.13 to 0.18 long, posterior to ovary. Vitelline glands short, compact, with close-set follicles, extending from posterior margin of ventral sucker to a little posterior to testes. Transverse vitelline ducts from both sides running centrally and joining immediately anterior to receptaculum seminis to form common duct; with no appreciable yolk reservoir. Uterus in the form of undulating descending and ascending coils, running between testes and extending posteriorly as far back as posterior end of body, provided at its termination with a metraterm approximately 0.4 mm long and extending posteriorly as far as posterior end of ventral sucker. Eggs numerous, 0.04 to 0.044 by 0.0184 to 0.023.

Host.—Ptyas korros. Location.—Intestine. Locality.—Rangoon.

Bhalerao's (5, p. 194) review of the genus Styphlodora does not appear to be too critical. The differentiation of the species on

the basis of "slightly oblique" and "distinctly oblique" testes where the two testes lie close together leaves much to be desired. Neither is the partial or complete separation of the testicular zones a reliable character, as the zones are subject to considerable variation with the contraction and expansion of the specimens during fixation. The three specimens obtained from Rangoon show all varying conditions: in one the testicular zones are well separated by a distance of 0.05 mm, in the other the posterior testis immediately follows the anterior, and in the third the anterior and posterior testes partially overlap. It thus appears that in matter of specific distinction no reliance can be placed on the difference of these two characters. S. dentipharungeata differs from all the species of Styphlodora in the presence of dentitions on the inner walls of the pharynx. The presence of a prepharynx in the present form further differentiates it from S. renalis, S. solitaria, and S. persmitus. Of the remaining species it resembles only S. nicolli, S. najæ, and S. bascaniensis in having the cirrus sac not contiguous with the ovary. Of these three forms the resemblance is closer to S. nicolli than to the other two species, the differences with the other two consisting chiefly in the extent of the cirrus sac and vitellaria. It differs from S. nicolli, in addition to difference in the structure of the pharynx, in the more limited extent of the vitellaria and in the shape of the testes and receptaculum seminis.

Subfamily RENIFERINÆ Pratt, 1903

Genus PTYASIORCHIS Mehra, 1937

Allopharynx STROM, Zool. Anz. 79 (1928) 167-172, in part.

Gogate (10) described as Ostiolum mehrai a trematode from the gall bladder and bile ducts of Ptyas korros and Ptyas mucosus in Rangoon. The only important character that differentiates Ostiolum from Pneumonæces is the absence of the longitudinal extracæcal folds of the uterus. Dollfus(8) on the question of the validity of the genus Ostiolum ignored, however, the importance of this character and considered Ostiolum synonymous with Pneumonæces. Ingles(14) and Caballero and Sokoloff(6) hold the same view as Dollfus, whereas Travassos and Dariba(26) recognize Ostiolum as a separate genus comprising the species O. medioplexus (Stafford, 1902), O. complexus (Seely, 1906), and O. coloradensis (Cort, 1917). The study of the ascenaing extracæcal uterine loops and their varying extent in Pneumonæces shows that all conditions may exist, from P. longiplexus (Staf-

ford, 1902) where the extracecal uterine loops extend from the posterior end of the body to near the anterior end, through P. variegatus (Rudolphi, 1819), where they reach the ovary and P. breviplexus (Stafford, 1902), where they extend up to the region of posterior testis, to P. uniplexus (Harwood, 1932), where they are very poorly developed and a short loop is present only on the left side of the body, extending only to the posterior margin of posterior testis. Mehra (1937), like other workers, doubts the validity of Ostiolum as a separate genus; but unlike them he thinks the character of the extracæcal uterine loops sufficiently constant to warrant the division of Pneumonæces into two subgenera, Ostiolum and Pneumonaces. In view of the fact that all stages of development of the longitudinal extracæcal folds of the uterus are present in Pneumonæces up to a stage where the loop is very poorly developed on only one side of the body, such as in *P. uniplexus*, it does not seem reasonable to stress this point as a character of generic or subgeneric importance, and in the absence of any other character differentiating these genera the author considers that Ostiolum should be regarded as a synonym of Pneumonaces. However, whether Ostiolum is regarded as a synonym or as a subgenus of Pneumonœces, Dollfus(8) and Mehra (1937) have rightly shown that Ostiolum mehrai does not belong to either, as it differs in a number of important characters, such as the position of the genital opening far behind the pharynx, that is, on the intestinal bifurcation, the presence of a moderately long esophagus, the presence of a comparatively large ventral sucker near the intestinal bifurcation, the small size of the receptaculum seminis, and the shape and size of the cirrus sac. Added to these is a difference in hosts and habitat: all species of *Pneumonaces* hitherto recorded are exclusively parasitic in the lungs of Anura, whereas the present material is from the gall bladder and bile ducts of colubrid snakes. On account of these differences Mehra created for Gogate's species O. mehrai a new genus, Ptyasiorchis, with P. mehrai as a type species. This new genus has a close affinity with Xenopharynx Nicoll, 1912, the points of resemblance being the general topography of the organs, the long intestinal cæca, the small size of the cirrus sac, the position of the genital pore, and the identical nature of the host and habitat. The chief differences between the two are in the structure of the pharynx and the distribution of the vitellaria and uterus. Strom(25) described a trematode, Xenopharynx (Allopharynx) amudariensis.

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from the gall bladder of a snake (Tropidonotus tesselatus) from Turkestan, which closely resembles O. mehrai of Gogate, the affinity being greater than that between Xenopharunx (Allopharunx) amudariensis and the other species of the genus. From other species of Xenopharynx, Storm's species exhibits differences in the structure of the pharynx, the position of the genital pore, and the distribution of the vitellaria and uterus. Mehra (1937) has rightly pointed out that Strom's species shows characters which do not permit it to be placed in Xenopharunx, and the latter name being quite inapplicable he has erected the genus Ophiorchis for its reception. Price(23) has rightly shown that the name Ophiorchis Mehra, 1937, is redundant, and the correct procedure is to elevate Strom's subgenus Allopharynx to the status of a genus. Thus Ophiorchis Mehra, 1937, becomes a synonym of Allopharynx. On account of the close similarity between O. mehrai Gogate, 1935, the type of the genus Ptyasiorchis Mehra, 1937, and Allopharunx, Price considers Ptyasiorchis Mehra, 1937, as a synonym of Allopharynx. Gogate's O. mehrai, though allied to Storm's Allopharynx, exhibits important differences which do not permit it to be placed in the same genus. It differs from Allopharynx in the position of the genital pore, the shape and extent of the cirrus sac, the absence of a metraterm, and the shape of the excretory bladder; and in these respects it resembles Xenopharunx. Its differences from Xenopharunx are also important, exhibited in the characters of the pharynx, vitallaria, and uterus. It thus appears to the author that Ptyasiorchis Mehra, 1937, a genus created for Gogate's Ostiolum mehrai, is distinct, and therefore cannot be considered identical with Allopharynx, a procedure adopted by Price. Table 2 emphasizes the distinctness of Ptyasiorchis Mehra, 1937, and shows its relationship with Xenopharynx and Allopharynx.

The diagnosis of the genus Ptyasiorchis Mehra, 1937, is as follows: Lepodermatidæ, Reniferinæ, Body moderately long and flattened. Oral sucker large, terminal or subterminal. Ventral sucker moderately developed. Prepharvnx short. Pharynx large and simple. Esophagus moderately long. Intestinal cæca ending a little anterior to posterior end of body. Genital pore anterior to ventral sucker, near intestinal bifurcation. Testes postovarian, entire, oblique, anterior testis lying partly or wholly behind middle of body. Cirrus sac weak, subspherical to oval, anterior to ventral sucker or just touching its anterior margin, enclosing a coiled vesicula seminalis and a pars prostatica. Ovary entire, much behind ventral sucker and at some distance anterior to testes, lying on median axis or deflected slightly laterally. Vitellaria in groups of follicles and with conspicuous vitelline ducts, extending from near level of intestinal bifurcation to close to end of intestinal cæca. Uterus voluminous, with descending and ascending transverse coils passing in between testes and extending posteriorly a little beyond intestinal cæca. Metraterm absent. Excretory bladder Y-shaped, with a subterminal pore and a long stem and two pronounced limbs reaching transverse vitelline ducts.

Table 2.—Comparison of Xenopharynx Nicoll, 1912, Allopharynx Strom, 1928, and Ptyasiorchis, 1937.

Characters.	Xenopharynx Nicoll, 1912.	Allopharynx Strom, 1928.	Ptyasiorchis Mehra, 1937.	
Pharynx	Peculiar, contiguous with oral sucker.	Simple, not contiguous with oral sucker.	Simple, not contiguous with oral sucker.	
Position of the genital pore.	Median, just over in- testinal bifurcation.	Median or slightly lateral, approximately midway between intestinal bifurcation and ventral sucker.	Median, just over intestinal bifurcation.	
Cirrus sac	Oval, anterior to ven- tral sucker.	Cylindrical, slender, extending for some distance or beyond ventral sucker.	Subspherical to oval, anterior to ventral sucker or just touch- ing its anterior margin.	
Vitellaria	Profuse in the neck region.	Absent in the neck region.	Absent in the neck region.	
Uterus	Not extending towards posterior end of bo- dy.	Extending towards posterior end of bo- dy.	Extending towards pos- terior end of body.	
Metraterm	Absent	Present	Absent.	
Excretory bladder	Y-shaped, with a long stem and two pronounced limbs reaching transverse vitelline ducts.	Imperfectly known. Probably a large vesicle with a slight indentation at the anterior end.	Y-shaped with a long stem and two pro- nounced limbs reach- ing transverse vitel- line ducts.	

Type species.—Ptyasiorchis mehrai (Gogate, 1935) Mehra, 1937.

Synonym.—Ostiolum mehrai Gogate, 1935.

PTYASIORCHIS MEHRAI (Gogate, 1935). Text fig. 2.

Ostiolum mehrai Gogate, Rec. Ind. Mus. 37 (1935) 455-458.

Body moderately long <sup>1</sup> and flattened, conical at anterior end but rounded at posterior. Sharp conical cuticular spines extending entire length of body, but distributed sparsely in poste-

<sup>&</sup>lt;sup>1</sup> Measurements are omitted from this account since they are in agreement with those of Gogate.

rior and dense in anterior part of body. Prepharynx short, inconspicuous in some specimens due to contraction of anterior part of body. Œsophagus moderately long, provided with musculature the extension and contraction of which brings a change in its length. Intestinal cæca with more or less irregular margins, extending a little anterior to posterior end of body. Ventral sucker approximately as large as oral but with less pro-

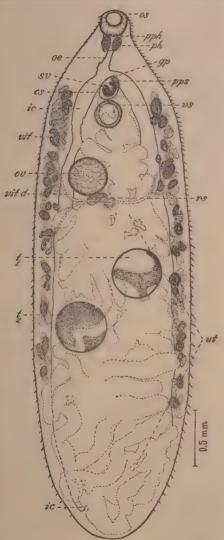


Fig. 2. Ptyasiorchis mehrai (Gogate, 1935). Ventral view.

nounced musculature and lying a little posterior to cæcal bifurcation. Genital pore at intestinal bifurcation. Testes and ovary spherical, appearing slightly oval in pressed specimens, the latter organ lying well anterior to the former. Testes entire, oblique, in separate zones, the anterior testis lying partly or wholly posterior to middle of body length and left of the median line. Receptaculum seminis pear-shaped and posterior to ovary. Vas deferentia run anteriorly dorsal to ventral sucker and near posterior margin of this organ unite to form the common vas deferens. Cirrus sac subglobular, weak, lying anterior to ventral sucker or just touching its anterior margin and enclosing for most of its space a much-coiled vesicula seminalis and a small pars prostatica. Ovary spherical, oval in slightly pressed specimens, lying usually a little to the right of the median axis well behind ventral sucker and much anterior to testes. Vitellaria in groups of closely compact follicles extending from level of intestinal bifurcation to near end of intestinal cæca. Longitudinal and transverse vitelline ducts usually conspicuous, the latter meeting posterior to ovary to form the yolk sac. Uterus very voluminous, with descending and ascending coils filled with ova and extending posteriorly to a little beyond termination of intestinal cæca. Metraterm absent. Excretory bladder Yshaped, with a subterminal pore and a long stem and two pronounced limbs reaching transverse vitelline ducts.

Host.—Ptyas korros; Ptyas mucosus. Location.—Gall bladder and bile ducts. Locality.—Rangoon.

### Family ACANTHOSTOMIDÆ Poche, 1925

Genus ACANTHOSTOMUM Looss, 1899

ACANTHOSTOMUM BURMINIS (Bhalerao, 1926).

Acanthochasmus burminis BHALERAO, Parasitol. 18 (1926) 4-13.

This species was described by Bhalerao(3) from Natrix piscator in Rangoon as Acanthochasmus burminis. The name Acanthochasmus was suggested by Looss in 1900 to replace Acanthostomum Looss, 1899, the latter being preoccupied (Insecta, vide Acanthostoma Krchbr.). This species has been recovered only from the water snake, and its absence from other snakes suggests that the intermediate host is some aquatic animal.

Host.—Natrix piscator. Location.—Intestine. Locality.—Rangoon.

## Family OMATOBREPHIDÆ Poche, 1925

Genus OMATOBREPHUS Nicoll, 1914

OMATOBREPHUS LOBATUM Mehra, 1928.

Omatobrephus folium THAPAR and ALI (1929).

Gogate (11, p. 455) once obtained two specimens of this parasite from *Ptyas mucosus*, killed in Rangoon. Gogate remarks that his specimens differ from the original forms described by Mehra (1928) from India in "(1) diminutive size; (2) the smaller size of the oral and ventral suckers; (3) the absence of a prepharynx; (4) the comparatively larger ovary; (5) the pear-shaped receptaculum seminis; and (6) the smaller ova". These differences have been considered by him due to individual variation. Whether an assemblage of these characters should be treated as individual variation or specific variation depends on a closer study based on a larger number of specimens, but in the absence of suitable material the author for the present reserves his judgment.

Host.—Ptyas mucosus. Location.—Intestine. Locality.—Rangoon.

# Family DICROCŒLIDÆ Odhner, 1910

Genus MESOCŒLIUM Odhner, 1911

MESOCCELIUM SOCIALE (Lühe, 1901).

Distomum sociale Lühe, Zentralbl. Bact. 30 (1901) 166-177. Mesocoelium meggitti Bhalerao, Ann. & Mag. Nat. Hist. IX 20 (1927) (1927) 611-615.

This form is commonly found heavily parasitising the toad (Bufo melanostictus Schneider, 1937) of Rangoon, and has been obtained only once from the present host. In the stomach of this host were found remnants of a large toad (probably Bufo melanostictus) which may have conveyed the present infection. The parasite has been recorded from various parts of the world, but the specimen obtained in Burma presents extreme variations in the body size, length of intestinal cæca, and arrangement of the genital organs. The intestinal cæca extend proportionately more posteriorly in smaller specimens than in the larger forms, and they range from approximately three-eighths to two-thirds of the body length; in rare cases the cæca are asymmetrical. Gonads usually spherical but sometimes oval. Testes

are very rarely either triangular, as described and illustrated by Lühe (16, fig. 5) in his original description, or bilobed; they are usually larger than the ovary; very rarely the reverse condition is seen. Right testis usually anterior to left, with ovary on right side of body just posterior to right testis; in a few cases the arrangement is reversed. Vitellaria consisting of a number of oval or round follicles, extending usually from side of oral sucker posteriorly along both sides of body to posterior extremity of intestinal cæca; in exceptional cases they may be sparsely developed and limited to one side of the body or alternatively be so profusely developed that they extend beyond the limit of the intestinal cæca. Ova 0.034 to 0.05 by 0.021 to 0.028 mm (a length of 0.0038 mm as given by Lühe in his original description is a misprint for 0.038). The presence of the variations enumerated above has given rise to the erroneous belief that the present form comprises more than one species. Bhalerao (4, pp. 611-615) described as M. meggitti a trematode from the intestine of a lizard, Mabuia dissimilis Hallow, 1857, which in all essential characters resembles M. sociale. The size of the body, the nature of the cuticle, the ratio of the suckers, the extent of the intestinal cæca, the cirrus sac and the vitellaria, the position of the gonads and of the receptaculum seminis in M. meggitti, fall within the limits of the variation in M. sociale already discussed. Bhalerao does not mention the presence of a prepharynx, but the material obtained by the author from the same host as that of Bhalerao shows a prepharvnx which, however, varies in length in proportion to the amount of contraction of the specimen. Thus it appears to the author that M. sociale, usually obtained from Bufo melanostictus, is the same as that from Mabuia dissimilis, though the systematic positions of the hosts are quite different. Animals which are so unlike may have something in common in their feeding habits, and this probably accounts for the presence of identical parasites in two dissimilar hosts. To corroborate this theory the author has caught both lizards and toads from his own compound and found on examination that both are infected with this parasite. The author has failed to find a clue to the intermediate host; in all probability it will be an insect the remnants of which are mostly found in the rectum of the infected animals. On several occasions ordinary grass blades with stems have also been found in the rectum of the infected toads. Various samples of this grass

were collected from localities where the toads are usually found, but so far no cysts have been obtained that can be ascribed to this species.

Host.—Ptyas mucosus. Location.—Intestines. Locality.—Rangoon.

## Family CYATHOCOTYLIDÆ Poche, 1925

Subfamily PROHEMISTOMINÆ Lutz, 1933

Genus GOGATEA Lutz, 1935

GOGATEA SERPENTIUM (Gogate, 1932). Text fig. 3.

Prohemistomum serpentum Gogate, Parasitol. 24 (1932) 318-320.

Gogate (10) described this parasite from the intestine of Natrix piscator as Prohemistomum serpentum. Lutz,2 in his revision of the family Cyathocotilidæ, created a subfamily Prohemistominæ, pointed out the difference between Prohemistomum and Gogate's form, and created a new genus Gogatea for the reception of the latter. Szidat accepted Lutz's view and considered Gogatea as a genus of Prohemistominæ, but considered the Prohemistominæ of Lutz and Szidat as a supersubfamily (Prohemistomida) and divided it into two subfamilies, Prohemistominæ and Szidatinæ, mainly on the ground of host differentiation, the former being all recorded from birds and mammals and the latter from reptiles. Apart from other considerations. the author can add to the numerous recognized cases of helminths in hosts belonging to groups other than those accepted as usual. In the present collection is a new strigeid, Mesostephanus burmanicus sp. nov., from a snake, Enhydris enhydris, all other species of this genus being reported from mammals or birds. This occurrence is in direct contradiction to the theory of host specificity as advocated by Dubois. The subfamily Szidatinæ, therefore, which is mainly distinguished from the other on host differentiation, appears to be redundant. Dubois (1938) erected a genus Szidatia for a parasite from Tropidonotus vipe-

<sup>&</sup>lt;sup>2</sup> Oberservatœs e consideracœs sobre cyacotylineas e prohemistomineas. Mem. Inst. Oswaldo Cruz. 30 (1935) 157-168.

<sup>&</sup>lt;sup>3</sup> Szidat, L. Parasiten aus Seeschwalben. 1. Ueber neue Cyathocotyliden aus dem Darm von Sterna hirundo L. und Sterna Paradisea Z. Parasitenk. 8 (1936) 294-299.

rinus, the metacercaria of which was described by Hughes (1929) as Diplostomulum joyeuxi Hughes, 1929, and the adult by Joyeux and Baer <sup>4</sup> as Prohemistomum joyeuxi. Szidatia closely resembles Gogatea, even in the character of the vitellaria which Dubois considers to be very different. The arrangement

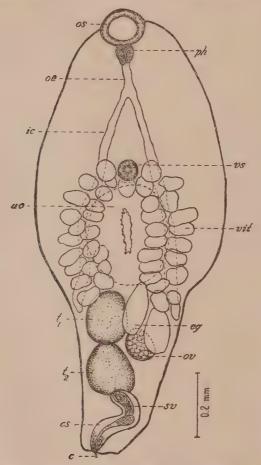


Fig. 8. Gogatea serpentium (Gogate, 1981). Ventral view.

of the vitelline glands, which in *Szidatia joyeuxi* extend even laterad to the adhesive organ, is also true for *Gogatea serpentium*: this is clearly seen in fresh and well-extended specimens.

<sup>\*</sup>Joyeux, C., and J. G. Baer. Sur un trematode de couleunre. Rev. Suisse Zool. Geneva 41 (1934) 203, 204.

The only difference in the vitellaria is the arrangement of the glands, which in the former are absent from the anterior margin of the adhesive organ, whereas in G. serpentium they extend along its anterior margin. This difference has not been emphasized by Dubois, nor is it of generic importance, and in the absence of any other important character the author considers both Szidatia joyeuxi and Gogatea serpentium as belonging to one and the same genus, the name of which has priority to the name Gogatea Lutz, 1935, including both. The foundation of genera on other than morphological characters would render identification impossible.

In a well-extended form body divided into an anterior region. oblong, lamelliform, or sublinguiform, and ventrally concave; posterior cylindrical, lodging sexual glands, cirrus sac, and distal portion of uterus. Gogate and Dubois describe the vitelline glands as confined to the dorsal side of the adhesive organ, but this condition is only true for contracted specimens. In a fully extended form vitellaria divided into large follicles disposed in two rows, arranged in the form of a horseshoe, encircling all margins of adhesive organ except posterior, and extending beyond this organ. Ventral sucker not embedded in adhesive organ as Gogate mentions, but quite conspicuous in fresh and wellextended specimens, and just anterior to adhesive organ. Genital organs well separated from adhesive organ, the condition described by Gogate holding good only for contracted specimens. Posterior portion of body similar to anterior part, showing all varying degrees of contraction. In extended condition testes well separated from one another by ovary, but in contracted state the two testes lying close together and ovary displaced slightly laterally. Intestinal cæca extending beyond adhesive organ, reaching posterior level of anterior testis or ending a little anterior to this level. Cirrus sac with internal vesicula seminalis, a well developed pars prostatica, and a small eversible cirrus. Eggs within uterus 1 or 2.

Host.—Natrix piscator. Location.—Intestine. Locality.—Rangoon.

### Genus MESOSTEPHANUS Lutz, 1935

MESOSTEPHANUS BURMANICUS sp. nov. Text figs. 4 and 5.

Body scoop-shaped, 0.85 to 1.26 mm long, divided into a broad anterior part, 0.73 to 1 by 0.36 to 0.46 mm, and a short, dorsally

directed appendix, 0.12 to 0.25 by 0.9 to 0.11. Oral sucker subterminal, 0.058 to 0.073 by 0.08 to 0.088 mm. Ventral sucker 0.034 to 0.088 by 0.04 to 0.45, just anterior to adhesive organ (0.18 to 0.22 by 0.19 to 0.24 mm). Pharynx 0.084 to 0.052 by 0.084 to 0.087. Œsophagus relatively long, 0.098 to 0.182. Intestinal cases slender, terminating a little anterior to junction of

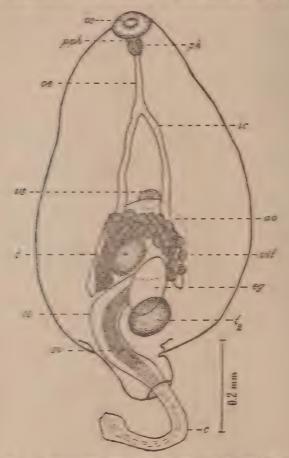


Fig. 4. Marchiegisonal farms has by bon. Entire Sinbal view

anterior and posterior parts of body. Genital pore at posterior end of body, subterminal and directed dorsally. Genital sinus well developed. Cirrus pouch 1.28 to 1.24 by 1.15 to 0.066, extending either to right or left around testes and every, its base lying in zone of anterior testis and containing a seminal vesicle.

a long pars prostatica, and an eversible cirrus. Testes oval, oblique, one behind the other, in separate levels; anterior testis 0.09 to 0.095 by 0.04 to 0.06 mm, on left side of body, posterior testis 0.09 to 0.095 by 0.07 to 0.09 mm, on right side of body. Ovary globular, approximately 0.05 to 0.06 mm in diameter, situated between testes. Vitellaria well developed, follicles forming almost a circle around adhesive organ. Uterus short, con-

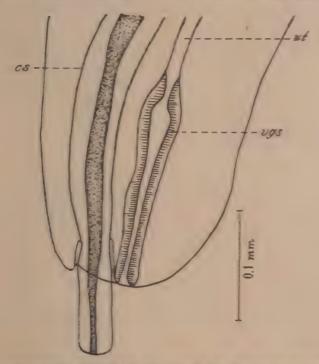


Fig. 5. Mesospephanus barmonious sp. nov. Posterior portion enlarged showing variation sphineter and cirros sae deflected.

taining 1 or 2 eggs. Vaginal spaineter well-developed. Eggs 0.098 to 0.14 by 0.073 to 0.096.

Host.—Enhydris enhydris.

Location.—Intestine.

Locality.-Rangeon.

Out of four species of Mesostephanus the present form resembles M. appendiculateides (Price, 1934) and M. appendiculatus (Ciurea, 1916) in having a small pharynx, a ventral sucker smaller than oral, and a few eggs in the uterus. It differs, how-

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ever, from both in having a long cophagus instead of a reduced one and a smaller number of eggs (1 or 2) instead of more (2 to 5), besides other minor differences in the size of the body parts. Dubois draws a sharp line between the strigeids of reptiles and those of birds and mammals. The occurrence of the present form in a reptile, when all other species of this genus are found in birds and mammals, is not in conformity with his views. In view of the persistence of the theory that particular helminths are confined to a definite group, and that similar forms in other groups are necessarily different species or genera, it is essential to emphasize that the author could not find in the material here described any morphological character or lack of characters which was not included in a definition of the genus Mesostephanus. He has no option therefore but to place it in that genus. In view of the large number (over 100) of specimens found several times in the host it is improbable that it was a pseudoparasite.

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# **ILLUSTRATIONS**

#### TEXT FIGURES

[Legend: ao, adhesive organ; e, cirrus; cs, cirrus sac; eg, egg; gp, genital pore; ic, intestinal cæca; æ, æsophagus; os, oral sucker; ov, ovary; ph, pharynx; ph d, pharyngeal dentition; pph, prepharynx; pps, pars prostatica; rs, receptaculum seminis; sv, vesicula seminalis; t1, anterior testis; t2, posterior testis; ut, uterus; vgs, vaginal sphincter; vit, vitellaria; vit d, vitelline duct; vs, ventral sucker.]

- Fig. 1. Styphlodora dentipharyngeata sp. nov. A, entire; B, pharynx enlarged.
  - Ptyasiorchis mehrai (Gogate, 1935). Ventral view.
     Gogatea serpentium (Gogate, 1932). Ventral view.

  - 4. Mesostephanus burmanicus sp. nov. Entire, dorsal view.
  - 5. Mesostephanus burmanicus sp. nov. Posterior portion enlarged, showing vaginal sphincter and cirrus sac deflected.

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# THYSANOPTEROLOGICA, IX

By H. Priesner

Of the Ministry of Agriculture, Cairo, Egypt

TWO TEXT FIGURES

#### 1. Genus IRIDOTHRIPS novum

Head heavy, much produced anteriorly, distance between antennal fossæ comparatively great; ocelli small, far apart, interocellar bristles situated at sides of front ocellus; mouth cone large, broadly rounded; maxillary palpi 3-jointed; antennæ 8-jointed, joints 3 and 4 with simple sense cones. Prothorax with one long bristle which is adpressed and directed backward at each fore angle, and two long bristles at each posterior angle; second posteromarginal bristle of pronotum largest; inner anteromarginals short. Bristles on forewing arranged on veins in uninterrupted rows. Abdomen normal, bristles at apex long, dorsals on tergite 9 short. Male brachypterous, female macropterous or brachypterous.

Genotype: Bregmatothrips iridis Watson.

Bregmatothrips iridis cannot remain in the genus Bregmatothrips, as the fore angles of the pronotum are provided with long bristles which have hitherto escaped notice. These bristles are usually adpressed and directed backward. The insect is closely allied to Frankliniella, particularly on account of the dense rows of bristles on the wings and the chætotaxy of the hind margin of the pronotum. It is distinguished from Frankliniella by the simple sense cones and the shorter antennæ.

## 2. Genus DIAPHOROTHRIPS Karny

Key to the subgenera of Diaphorothrips Karny.

- a. Postocellar bristles long, hairlike; anteocellars small (type, unguipes Ka.) Subgenus Diaphorothrips s. str.

  a. Postocellar bristles small; anteocellars moderately long (type, hamipes Ka.) Subgenus Cnemidothrips nov.
- DIAPHOROTHRIPS (CNEMIDOTHRIPS) CLAVIPES sp. nov.

Female.—Blackish brown to black, antennæ and legs wholly dark, tarsi yellowish gray, or fore tarsi grayish yellow to yellow,

antennal joint 2 and extreme base of 3 somewhat paler yellowish gray; body bristles dark, cephalic spines on genæ black; wings hyaline in basal half, distinctly smoky in apical half or more, longitudinal streak not well developed; fringe paler in basal half of wings than in apical. Major bristles of abdomen yellowish, anal hairs shaded basally.

Head length from eyes 415 µ, including interantennal projection 440 μ, width across eyes about 286 μ, behind eyes 280 μ; very short tempora discernible behind eyes, eyes very moderately large, occupying fore angles of head, somewhat more strongly produced ventrally, their hind margin nearly straight, lateral diameter 102 to 106 u, cheeks behind eyes about 330 u long, almost parallel-sided, hardly noticeably convex, somewhat more strongly constricted near base; cheeks with six to eight dark spines, longest spine 35 to 40 u; mouth cone long and slender, rounded at apex, labrum somewhat protruding; hind ocelli situated in or somewhat in front of middle of eyes, about 83 u distant; anteocellar bristles moderately long, 39 to 47 µ; postocellars very short, 28 μ; postoculars very long, 256 to 276 μ, pointed, very close (12 to 16 \(\mu\)) to eyes, situated behind inner margin of latter, head not produced in front; antennæ about 830 µ long, slender. Measurements of joints: 59 (55), 91 (47), 138 (51 to 53), 150 (50), 140 (43), 95 (34), 79 (28), 75 to 79 (20) u: joint 1 nearly parallel-sided, joint 3 straight laterally. transversely wrinkled at basal third, well provided with setæ as is joint 4, joints 5 to 7 somewhat obliquely truncate apically, joint 8 fusiform, strongly constricted at base; sense cones weak; dorsals, joint 3 with 2 (an outer and an inner), joint 4 with 4, joint 5 with 1 + 1, joint 6 with 1 + 1, joint 7 with 1. Pronotum strongly emarginated at somewhat thickened fore margin, hind margin moderately convex, 208 to 216 y long, 502 or (with coxæ) 580 u broad; coxæ triangular; interior anteromarginals of pronotum conspicuous but moderately long (40 μ), anteroangulars about 60 μ long; prothorax with strong, dark, endothoracic median line; posteroangular bristles well 138 μ; coxals about 83 u: bristles rounded at tip; fore femora enlarged (about 215 µ thick), in middle of exterior margin with an 87-u-long bristle; fore tibiæ with an inner subapical distinct toothlike process, which protrudes from inner margin of tibia for about 28 to 32 µ; fore tarsi with strong, curved tooth. sonotum with faint, transversely netlike, metanotum I with polygonally netlike structure, the latter with two micropores and two small setæ behind them. Wings normal, with 34 or 35 duplicated cilia, basal wing bristles nearly pointed, about 50, 75 to 80, and 83 to 87  $\mu$  long, respectively, partly shaded. Legs with only pale preapical hair of tibiæ long. Abdomen slender, segment 1 distinctly netlike, sculptured above; bristles at sides of segments yellowish, longest on segment 6, about 268  $\mu$ , on segment 7, 244  $\mu$ , on segment 8 much shorter, 118 to 160  $\mu$ ; bristles on segment 9 long, bristle 2 about 485  $\mu$  long; tube long (dorsally 588  $\mu$ ), decidedly longer than head, breadth at base 162, at tip 60  $\mu$ , slightly narrowed towards apex, somewhat more strongly narrowed about apical third; anal hairs weak, about 260  $\mu$  long.

Riouw Archipelago, Doerian, September 1933, 1 female, K. Dammerman No. 45.

This species differs from D. (C.) hamipes Karny by its larger size, the stronger lateral spines, the strongly shaded wings in their apical half, and especially by the longer and slenderer, wholly dark antennæ; in hamipes joints 3 and 4 of the antennæ are 87(43) and 95(46)  $\mu$  long (broad), respectively. I have also seen D. hamipes from Sumatra (Medan, May 15, 1922) where it was collected by L. Fulmek in dry pods of C wealpinia pulcherrima.

### 3. Genus MYOPOTHRIPS novum 1

Head short, a little longer than broad, cheeks strongly convex; eyes large, oval; antennæ 8-jointed, moderately long, joint 8 broad at base; mouth cone short, moderately broadly rounded, middle of prosternum little surpassing, labrum bluntly pointed; one pair of short postocular bristles present; surface of vertex not reticulated. Pronotum little shorter than head; fore femora of female enlarged, fore tibiæ interiorly at apex with a stout, hooklike tooth; fore tarsi short, toothless, but with well-developed claw; anteromarginal bristles of pronotum small, posteroangulars long, pointed. Wings broad, not narrowed, with duplicated cilia. Tube normal, about as long as head, bristles on segment 9 shorter than tube.

Genotype: M. symplocobius sp. nov.

This genus is certainly more closely allied to *Smerinthothrips* and *Teuchothrips* than to *Diaphorothrips*. The armature of the forelegs is distinctive.

## MYOPOTHRIPS SYMPLOCOBIUS sp. nov.

Female.—Black, fore femora paler (brownish yellow) apically, fore tibiæ brownish yellow, shaded at exterior margin, all tarsi yellow, scarcely or but slightly shaded; antennal joint 1 dark, joint 2 dark only at extreme base and at interior margin, otherwise yellow or ochreous, joints 3 to 5 clear yellow, joint 6 yellow, shaded at apical margin, joints 7 and 8 dark, joint 7 somewhat paler basally (brownish yellow); all prominent bristles of body dark; wings shaded throughout length with scarcely noticeable median longitudinal darkening.

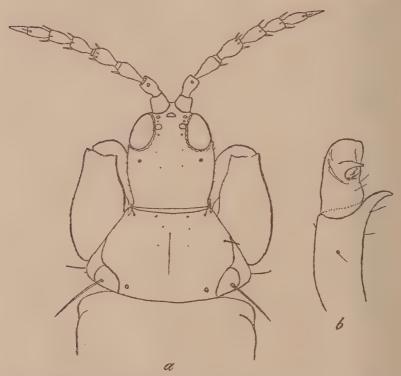


Fig. 1. Myopothrips symblocobius gen. et. sp. nov. a, female; b, apex of fore tibla and tarsus of female, viewed from below.

Head length from eyes 236  $\mu$  (including interantennal projection 264  $\mu$ ), breadth behind middle 217 to 220  $\mu$ , much narrower across eyes; cheeks evenly and strongly convex, constricted posteriorly; eyes large, oval, lateral diameter 87 to 91  $\mu$ , dorsal (longest) diameter 99 to 102  $\mu$ , evenly rounded at hind margin, somewhat shorter ventrally than dorsally; hind ocelli situated in front of a line drawn across middle of eyes and attached to them; one microseta in front of, and behind, hind

ocelli: pores of postocular bristles 24 μ from eyes, 28 μ from cheeks, bristles directed upward and most likely short and thick; head rugose, its sculpture consisting of cross wrinkles, set with fine microsetæ: mouth cone as indicated above; a small pale dot at sides of front ocellus. Length of antennæ 433 to 450 μ; measurements of joints: 43 to 47 (base, 45; tip, 35) 59 (35) or 36), 67 (35 or 36), 65 to 67 (41), 59 (36), 57 (33), 55 to 59 (28), 45 (16 or 17) u; joint 1 somewhat narrower at tip, joint 2 with large transverse areola before apex, joint 3 somewhat transversely wrinkled basally, abruptly constricted before apex. joint 4 heavy, strongly convex, joint 8 broadest at base, somewhat narrower at base than joint 7 at apex; joint 3 with 1 (exterior) sense cone, joint 4 with 1 + 2 +1 sense cones, joints 5 and 6 with  $1 + 1^{-1}$  each, joint 7 with 1 dorsal. Pronotal length 217 to 220 µ, breadth about 345, including coxæ about 398 u; anteroangular bristles very moderately developed (24 to 28 u?), anteromarginals minute; posteroangulars long, curved, pointed, 118 to 126 u; coxal bristle blunt, 47 u; anterior margin of pronotum almost straight, hind margin convex. Fore femora enlarged, fore tibiæ stout, with an inner, hooklike, yellow tooth at apex; fore tarsi short, unarmed, claw prominent. Length of pterothorax 467, width 450; fore angles of mesothorax rounded. Legs without prominent bristles. Wings broad (above 118 u in middle), of equal width throughout, somewhat curved, with 15 to 18 duplicated cilia; basal wing bristles pointed, bristle 3, 75 µ long. Abdomen as in many Smerinthothrips species with stout, dark (clear at tip) pointed, lateral bristles. Tube somewhat narrowed behind base and again at apical third, length (laterally 256 µ, width across base 102 μ, at apex 43 μ; bristles at sides of segment 7 158 μ, at segment 8 122 u, segment 9, bristle 1, 146 to 158 u, bristle 2, 110 to 118 µ, shorter than tube; lateral anal hairs about 158 µ.

Male unknown. Larvæ similar to those of *Smerinthothrips*. Java, Tjisaroewa, near Buitenzorg, altitude 1,000 meters, July 26, 1925, in leaf gall of *Symplocos javanica* Kurz, 2 females and larvæ (W. Docters van Leeuwen).

### ANDROTHRIPS KUROSAWAI sp. nov. Text fig. 2.

A large species with very much enlarged fore femora. Brown to ochrous, probably somewhat faded, darkest specimen with tube of darker brown, paler towards apex; middle and hind femora of same color as body, fore femora yellowish at

apex; all tibiæ and tarsi and also joints 3 to 6 of antennæ pale yellow. Forewings faintly shaded with yellow, hind wings with a light gray longitudinal stripe which comes close to hind margin at apical half of wing.

Head longish, cheeks distinctly narrowed towards base, straight or even very slightly concave, somewhat rugose under high magnification, on account of dense transverse striation; eyes large, tempora behind eyes somewhat angular; ocelli large, touching eyes, hind ocelli close to each other; one microseta close to front ocellus, another microseta and a porus exteriorly in front of hind ocelli; two microsetæ, one behind the other, after hind ocelli; mouth cone reaching middle of prosternum, rounded. Antennæ slender, apical joints thin, sense cones tender, two on joint 3, four on 4. Prothorax strongly widened posteriorly, fore and hind margin nearly straight, coxæ rounded, bristles rather pale, long, knobbed, bristles in exterior fore angle very long, interior bristles vestigial. Fore femora very broad, flattened, with distinct, narrow, parallel-sided tooth at base; interior margin of fore femora straight, with several irregular rows of very fine rounded tubercles the size of which does not exceed 4 µ; fore tibiæ curved, with a flat, small plate apically within; fore tarsi with a strong, forwardly directed tooth which emerges from a broad base. Basal wing bristles long, all knobbed; 10 to 15 duplicate cilia. Tube shorter than head; anal hairs long, bristles on segment 9, bristles 1 and 2 knobbed, bristle 3 pointed.

Head, length 288 μ, including interantennal projection, 313 μ; width across eyes, 217 u; across cheeks behind eyes, 220 u; lateral diameter of eyes, 102 µ; inner distance of hind ocelli, 26 μ; distance of postocular bristles from eyes, 24 to 26 μ; length of postoculars, 130 to 138 µ; length of antennæ, 519 to 554  $\mu$ ; antennal joints, 36 (bristle 47), 59(30), 87(41), 95 to 99(35 to 37), 77(26), 67(24), 59(21), 43 to 47(14). Length of pronutum about 233 μ, width without coxæ, 433 μ; including coxæ, 485 μ; anteroangular bristles, 99 μ; laterals, 118 μ; posteroangulars, 130 to 138 u; width of fore femora, 260; length of basal tooth, 32 to 40. Width of pterothorax, 519 to 536 u; length of wings, 133 μ; lengths of basal wing bristles, 95, 118, and 158 µ; length of hind tibiæ, 346 µ. Tube, length about 208  $\mu$  (dorsal 190); width across base, 97  $\mu$ ; at apex, 47  $\mu$ ; lateral anal hairs, 295; bristles on segment 9 (paratype); bristle 1, 197  $\mu$ ; bristle 2, 205  $\mu$ ; bristle 3, 205  $\mu$ .

Male.—A single male was contained in the same material, which, though much smaller than the females, agrees in the armature of forelegs (femora less stout), and in the color, except that antennal joint 6 is shaded apically and the apices of antennals 5 and 6 are slightly shaded. Double fringe, 9 to 10. Measurements of male (allotype): Head length, 233  $\mu$ ; including interantennal projection, 256  $\mu$ ; width across eyes,

181 μ; lateral diameter of eyes, 90 μ; postocular bristles, 95 to 102 μ; antennal joints, 3, 71 (36), 4, 79 (36), 5, 69 μ. Length of pronotum, 197 μ; posteroangular bristles, 95 to 99 μ. Basal wing bristles, 65, 85, 122 to 126; width of pterothorax, about 400. Bristles on segment 9, bristle 1, 197 (blunt); bristle 2 (spine), 39; bristle 3 (pointed), more than 197. Tube length, lateral, 185 μ; dorsal, 173 μ; width across base, 75 μ; at apex, 37 μ; anal hairs, 235 μ.

LUZON, Laguna Province, Los Baños, July 30, 1929, sweeping material, collected by Dr. T. Ishii, communicated to me by Mr. M. Kurosawa to whom I have the

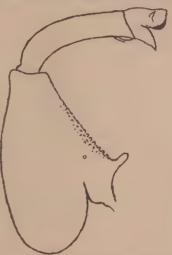
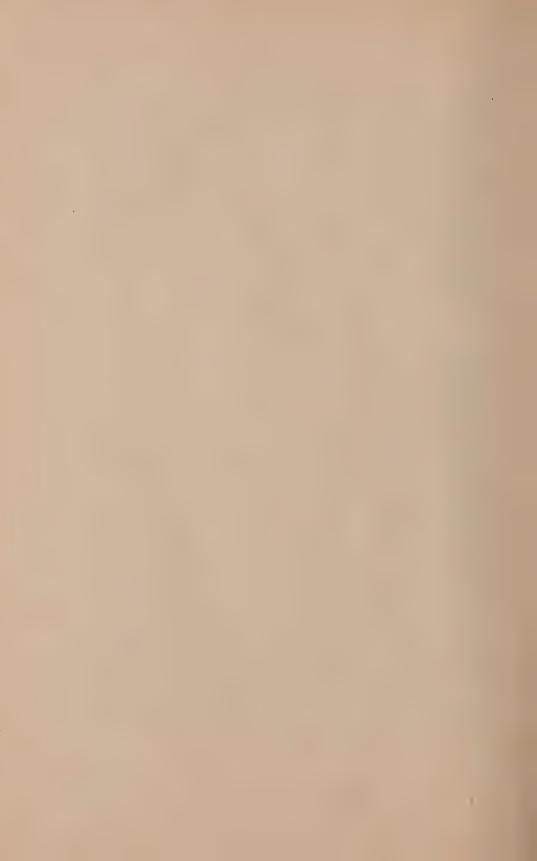


Fig. 2. Androthrips kurosawai sp. nov. Foreleg of female.

Kurosawa to whom I have the pleasure of dedicating the new species.

This insect cannot be easily confused with any of the species hitherto known, as Androthrips collaris Karny, A. ochraceus Karny, and A. flavipes Schm. are distinct by their coloration; A. ochraceus, specimens of which I was able to compare, having interior margin of fore femora not set with tubercles, and the whole insect being smaller; A. obscuratus Priesner and A. ramachandrai Ramakrishna Ayyar have middle and hind tibiæ dark; A. coimbatorensis Ramakrishna Ayyar has basal tooth of fore femora much larger and broadly triangular, no tubercles on fore femora, stouter antennæ, and only about five double fringe hairs; A. flavitibia Moulton is smaller, has shorter antennæ, and is, after Moulton, allied with flavipes, having thus apparently no femoral tubercles; A. melastomæ Karny has a series of more conspicuous tubercles (small teeth) at the fore femora within, and is a much smaller insect.



# **ILLUSTRATIONS**

### TEXT FIGURES

- Fig. 1. Myopothrips symblocobius gen. et. sp. nov. a, female; b, apex of fore tibia and tarsus of female, viewed from below.
  - 2. Androthrips kurosawai sp. nov. Foreleg of female.

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### BOOKS

Books reviewed here have been selected from books received by the Philippine Journal of Science from time to time and acknowledged in this section.

#### REVIEWS

Your Puppy and How to Train Him. By Huldine V. Beamish. New York, Lee Furman, Inc., 1938. 206 pp., illus. Price, \$2.

This book is divided into eighteen interesting chapters dealing with: the question of intelligence, buying a puppy, first lessons, early discipline, civilizing a puppy, free work and preliminary retrieving, early retrieving hints, communal training and signals. relationship with other animals, jumping and advanced work, foods and feeding, the bitch, mating and whelping, rearing puppies, the orphan, ailment in brief, the scourge of terror, and shows and showing, besides an introduction and a conclusion. There are excellent plates illustrating the different tricks a dog can acquire by proper handling and training. Although there are some loose statements, such as "some cow dung or chip droppings—inevitable temptation to puppies, and the surest way to develop worms" and the theories given on the cause of canine hysteria which seem to be obsolete (the disease being now known to be traceable to vitamin B deficiency), the work is a useful contribution. The book is written in a very convenient, attractive and readable form. It is primarily intended for those who wish to give a puppy a break to proper dog education not to become "a mere dog" but to become a well-behaved and useful pet.—L. M. Y.

The Mineral Resources of the Philippines for the Years 1934-1938. Part I. Gold Mines. By William F. Boericke and Nestorio N. Lim. Department of Agriculture and Commerce Technical Bulletin 13. Manila, Bureau of Printing, 1939. 135 pp., illus. Price, ₱1.30, postpaid.

The Bureau of Mines has just published Part I of a new volume entitled "The Mineral Resources of the Philippines, 1934–1938", devoted to a description of the gold mining industry of the Commonwealth. On account of the importance of this industry, the present volume is concerned entirely with the gold mines.

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The new volume takes up the development in gold mining since 1934, and appears in the same general form as previous publications by the former Division of Mines, under the Bureau of Science, under the same title. It contains five maps, showing the five principal gold-producing areas in the Philippines with the location of all the principal properties, and several graphs which present the growth of gold production and afford easy comparison between production here and in the United States. It includes descriptions of mining conditions in the Mountain Province, Camarines Norte Province, Masbate Province, Surigao Province, and Central Luzon. Every important producing mine, as well as many semideveloped properties, are described individually from actual field inspections made by the authors, W. F. Boericke and N. N. Lim. mining engineers on the staff of the Bureau of Mines, in cooperation with other Mines officials. Tabulations of gold production of the various producing mines, as well as of the several mining areas, add to the value of the

This is the most comprehensive review of the gold mining industry that has ever been prepared by the Bureau of Mines, and will no doubt be welcomed by mining engineers, investors, and those who wish to obtain authentic information on the present progress of local gold mines.—Q. A. A.

Handbook of Fertilizers; Their Sources, Make-up, Effects and Use. By A. F. Gustafson. New York, Orange Judd publishing Co., 1939. 172 pp., front., illus. Price, \$1.75.

The use of natural fertilizers in agriculture has long been known and practiced. However, artificial fertilizers are of comparative recent introduction. The need for adequate knowledge of their sources, compositions, applications, and effects is therefore most vital in the restoration of soil productivity. This is especially true in the old sections of the Philippines, where the soil has been depleted of its fertility on account of its being continuously cropped from generation to generation practically without returning to it, in the form of fertilizers, any of the plant foods removed by the successive crops. Farming today, to be successful, requires that the farmer be in possession of adequate and up-to-date knowledge of fertilizers. The appearance of Doctor Gustafson's "Handbook of fertilizers; their sources, makeup, effects and use", a very practical, clear, concise but complete body of information on this vital factor of present-day farming, is very timely.

The book contains all that is essential to the farmer, gardener. or fertilizer dealer about fertilizers. Moreover, the sequence of its contents is very logical. The book opens with a chapter on plant growth requirements, which leads to a readable discussion on the story and utilization of fertilizers. This chapter is followed by separate chapters on the three more important fertilizer elements; nitrogen, phosphorus, and potassium, and their sources. composition, and manufacture, use, and the effects of each of which are briefly discussed. The chapter is very important, as the visible effects described, especially those on the plant, are the only means by which the average farmer can tell, more or less, the fertilizer needs of his soil. Information on pertinent government requirements for effective factory-mixed fertilizers constitutes another chapter and shows how far the government has gone in protecting the farmer. Another interesting section of the book is a discussion on home-mixed fertilizers, which consists of several formulas and specific directions for mixing the ingredients. The purchase and extent and methods of application of fertilizers, with arguments pro and con in the next chapter, are food for thought.

Owing to the rôle that it plays especially in acid soils, lime is taken up quite in detail. Its functions, sources, applications, and effects are clearly noted. Finally the book closes with a very brief chapter on organic matter, its importance and relation to fertilizer practice, and the methods of maintaining it in the soil.

The book is written in a popular style with hardly a technical term that is not explained and made understandable to the layman. It is especially valuable because of the many useful and instructive tables and illustrations. Farmers, teachers of agriculture, especially of gardening, and fertilizer dealers will find this book very useful and convenient.—A. S. A.

An Outline of General Forestry. By Joseph S. Illick. Third edition, revised and enlarged. New York, Barnes & Noble, Inc., 1939. 297 pp., illus. Price, \$1.

This compact book gives a comprehensive and systematic outline of general forestry, with its problems and their solutions. Its thirty-one concise chapters, appropriately divided, impart to both the student of forestry and the layman the outstanding principles of forestry knowledge, and clearly portray "what Forestry is, how it is developed, what it is doing, where it is heading, why it is needed, and what benefits it is bringing to

mankind". One outstanding feature of the book is the effective use of illustrations and graphs which show at a glance comparative figures relative to a particular topic under discussion. At the end of each chapter is a list of selected references for the convenience of the reader who may wish to delve further into the subject dealt with in the chapter.—T. N. R.

The Etiology of Trachoma. By Louis A. Julianelle. New York, The commonwealth fund, 1938. 248 pp., front., illus. Price, \$3.25.

This monograph presents a critical and analytical review of the concepts of the etiology of trachoma that have been promulgated up to the present day, and evaluates the results of the research carried on at the laboratory of Washington University and elsewhere. The author first discusses the clinical aspects of trachoma to be sure that his materials are from real cases of trachoma. He then reviews the epidemiology, general conditions in the etiology and infectivity, and the microörganisms associated with trachoma.

After pointing out some defects in the experiments of other investigators, particularly the lack of controls, the small number of trials, the differences in infectivity of the material, and disregard of animal susceptibility, he describes and analyzes carefully the well-controlled, very numerous experiments on the etiologic agent of trachoma performed in his laboratory.

The evidence now available no longer permits an impartial observer to deny that the disease is an infection in its own right without benefit of associated factors. The experiments on the testicular and cerebral passage conducted by the author and his assistants clearly demonstrate that the infectious agent can be recovered in the absence of visible and cultivable organisms. This finding strengthens the viral concept of the etiology of trachoma.

The author summarizes the facts on the etiologic agent of trachoma as shown in his experiments of low infectivity, occasional filterability, marked tissue especialization, ineffectual immunogenic properties, slight propagative capacity, and sensitivity to deleterious agents. The infection it causes is accompanied by inclusion bodies. All these properties suggest the probability that the infectious agent is a virus. What remains to be determined by further investigation is whether the virus is synonymous with the inclusion bodies or its component elements and whether the elementary body may be interpreted as a rickettsia.

The attempts by the author to cultivate the inclusion body in tissue culture have given negative results. It is beginning to appear that viruses and their inclusion bodies may be actually the same thing.

The monograph contains thirteen illustrations, including ten plates. Its bibliography is classified and exhaustive. It is highly recommended for ophthalmologists and will be serviceable to bacteriologists and pathologists.—G. de O.

Modern Methods of Refining Lubricating Oils. By Vladimir A. Kalichevsky. American chemical society monograph series no. 76. New York, Reinhold publishing corporation, 1938. 235 pp., illus. Price, \$6.

The use of solvent extraction processes in the commercial refining of lubricating oils is of recent development. These processes, which employ numerous kinds of solvents, are so many that some sort of systematic classification is necessary to facilitate future development along each line. To this end this book has sufficiently served its purpose.

The various topics discussed in this book include methods of evaluating lubricants by certain properties of the refined oil; comparative studies of the old sulphuric acid treatment and the more modern solvent extraction methods of refining; an extensive discussion on the classification of the various solvents used in the purification of lubricants; and general principles of solvent refining processes, including different commercial refining methods.

The book gives a comprehensive discussion of the so-called "additives or oil improvers" such as pourpoint depressants, viscosity index improvers, oiliness carriers, oxidation inhibitors, and many other topics of interest.

Although a great portion of the book is devoted to discussions of general principles underlying the refining of lubricating oils, to those who desire to go into details the book offers quite a good number of references after each chapter. A list of patents is appended to the text.—I. P.

The 1938 Year Book of Physical Therapy. Edited by Richard Kovács. Chicago, The Year Book Publishers, Inc., 1938. 486 pp., illus. Price, \$2.50.

This 1938 Year Book of Physical Therapy contains two parts. The first half deals mostly with physical therapy methods, giving the reader an idea of the different kinds of physical energies used together with their respective basic foundations. The

other half deals with the practical applications and therapeutic indications of these physical agents in the treatment of various diseases. As a whole this is a fine reference book not only for the specialist in this line but also for every practitioner who cannot practice medicine and surgery without the valuable aid offered by physical therapy.—P. S. C.

Palæozoic Fishes. By J. A. Moy-Thomas. New York, Chemical publishing Co., Inc., 1939. 149 pp., front., illus. Price, \$2.

This book is useful to zoölogists working on comparative anatomy and evolution. To a certain extent it may be useful also to palæontologists interested in Devonian stratigraphy. It traces the changes undergone by fishes in their evolutionary history, beginning with the jawless ostracoderms of the early Ordovician, and winding up with the direct ancestors of the modern teleosts. Every known fish fossil is illustrated and described in detail. The materials are arranged in a natural sequence and discussed in a unique manner so as to bring forth their relationships. The anatomical changes are followed from one species to another, giving notations where one line of development branched off, and where another species is developed.

A good list of references, a glossary, and a detailed index form the last part of the book.—G. L. A.

Practical Identification of Endoparasites for Veterinarians. By John H. Whitlock. Minneapolis, Minnesota, Burgess Publishing Company, 1938. 37 pp., illus. Price, \$1.25.

This publication is an attempt at simplifying the complicated natural classification of the endoparasites of domesticated animals. The method of identification followed in this booklet is so simple that an average veterinarian can, with some degree of accuracy, identify specimens of endoparasites. It should serve as an adjunct to the laboratory guide in veterinary helminthology for veterinary students. However, it is of very limited use to veterinary practitioners, contrary to the claim of the author. While this booklet would certainly serve as a valuable guide for government field veterinarians and veterinary meat inspectors in the identification of endoparasites encountered in autopsies and meat inspections, it should not, except in very rare cases, serve as a guide in the accurate diagnosis of parasitic diseases in vivo, because in such cases the veterinary practitioners must depend upon the accurate identification of the ova and larvæ of the different species of endoparasites which are

found in the fæces, urine, vomitus, phlegm, and blood of infested animals.—Z. J.

#### RECEIVED

- BOERICKE, WILLIAM F., and NESTORIO N. LIM. The mineral resources of the Philippines for the years 1934-1938. Part I: Gold mines. Department of Agriculture and Commerce technical bulletin 13. Manila, Bureau of Printing, 1939. 135 pp., illus. Price, \$\P\$1.30, postpaid.
- Forbes, Russell. Purchasing for small cities. Chicago, Public administration service, 1939. 22 pp., illus. Price, \$0.50.
- Hosiery dyeing and finishing. A practical informative reference book for manufacturers and merchandisers of all types of hosiery products, outlining progress and production in this important industry. Compiled by the technical staff of Onyx oil & chemical company, Jersey City, N. J., 1939. 128 pp., illus.
- The Journal of Endocrinology, vol. 1, no. 1, June, 1939. London, The Oxford university press. Subscription rate per volume, 30 s., or \$6 in U.S. currency.
- Kurtz, Earl Nicholas. Woman, that eternally supreme question answered. The mystery of truth made startling in its simplicity: "Truth is stranger than fiction". Boston, Meador publishing co., 1938. 437 pp. Price, \$2.50.
- MAUERSBERGER, HERBERT R., and E. W. K. SCHWARZ. Rayon and staple fiber handbook. A practical reference book for the producer, manufacturer, processor, distributor, drycleaner, launderer, economist and student. 3d enl. ed. New York, Rayon handbook company, 1939. 832 pp., illus. Price, U. S. & Canada, \$4.50; Foreign, \$5.75.
- MIKHAILOV, NICHOLAS. Land of the soviets. A handbook of the U. S. S. R. Translated from the Russian by Nathalie Rothstein. New York, Lee Furman, Inc., 1939. 351 pp., front., illus., maps. Price, \$2.50.
- MONTGOMERY, WILLIAM J. Theory and practice of mine ventilation. Columbus, Ohio, The Jeffrey manufacturing co., 1936. 285 pp., front., illus. Price, \$3.50.
- Moy-Thomas, J. A. Palæozoic fishes. New York, Chemical publishing co., inc., 1939. 149 pp., illus. Price, \$2.
- Physicians' vitamin reference book presenting to the clinician a useful compendium of the latest facts about vitamins by the medical division, professional service department, E. R. Squibb & sons. Second edition, revised. New York, E. R. Squibb & sons, 1938, 160 pp., illus.
- Transactions of the American institute of mining and metallurgical engineers (incorporated) volume 132. Petroleum development and technology 1939. Petroleum division. Papers and discussions presented before the division at meetings held at San Antonio, Oct. 5-7, 1938. Los Angeles Oct. 20-21, 1938; New York, Feb. 13-16. 1939. New York American institute of mining and metallurgical engineers (incorporated), 1939. 625 pp., illus.



## ERRATA

#### VOLUME 68

Page 10, line 16, for symbiform read cymbiform.

Page 63, line 26, for Neiuwenhuisi read Nieuwenhuisi.

Page 64, line 1, for Claudopodanthus read Cladopodanthus.

Page 74, omit lines 34, 35, and 36 and substitute the following: This species may be distinguished from *Octoblepharum* in the field by the narrower, more brittle leaves. In any event the

Page 79, line 20, for 44. read 4.

Page 87, line 35 should read: appears in the mountains of Luzon.

Page 91, line 3 from below, for mollucense read moluccense.

Page 93, line 4 from below, for gragarious read gregarious.

Page 95, lines 30 and 31, for mollucense read moluccense.

Page 96, lines 17 and 39, for mollucense read moluccense.

Page 103, lines 1, 2, 10, and 12, for Mercyopsis read Merceyopsis.

Page 117, line 16, for *subulata* read *Steerei*; line 18, for Barbula subulata Broth. read Barbula Steerei Bartr. nom. nov. (Mr. William C. Steere has called my attention to the fact that the name B. subulata Broth. is antedated by B. subulata P. Beauv. I take pleasure in renaming this species for Mr. Steere as indicated above.)

Page 126, line 9, for fragil read fragile.

Page 181, line 31, for Micromitrium read Macromitrium.

Page 203, line 29, for Endotrichiella read Endotrichella.

Page 207, line 24, for idenitcal read identical.

Page 210, line 13, for rectagular read rectangular.

Page 262, line 7, for laxt read lax.

Page 277, line 29, for Lepidium read Lopidium.

Page 279, line 32, for Synoicuos read Synoicous.

Page 290, line 2 from below, for thing read thin.

Page 302, line 1, for Dimunitive read Diminutive.

Page 309, line 7, for Dizon read Dixon.

Page 319, line 16, for regida read rigida.

Page 376, lines 33 and 37, for Pylasia read Pylaisia.

Page 399, line 13, for Sinouse read Sinuose.

Page 403, line 22, for euxydictyon read eurydictyon.

Page 405, line 22, for refuscens read rufescens.

Page 406, line 28, for mollucense read moluccense.

Page 407, line 1, for Meryopsis read Merceyopsis; line 38, for Barbula subulata Broth. read Barbula Steerei Bartr.

Page 426, line 8, for subulata Broth. read Steerei Bartr.

#### VOLUME 70

Page 8, line 14, omit Do.

Page 10, Fig. 1, for Antenal grove read Antennal groove.

Page 12, line 20, for ceiling read coiling.

Page 13, line 12 from below, for or read for.

Page 14, line 4 from below, for with antepygidial bristles read without antepygidial bristles.

Page 15, line 17, for with lateral read with none or one lateral.

Page 17, line 7, for female read male.

Page 22, below Fig. 9 add the following note to the subgenus *Nosopsyllus*: As the paper was completed July 1, 1937, the description of *Nosopsyllus nicanus* Jordan, 1937, which appeared in Nov. Zool. XL: 295-296, is not included in this text.

Page 23, line 2, for third read fourth; line 4 from below, for laeviceps Wagner read laeviceps ellobii Wagner.

Page 24, line 22, for two bristles read two stout bristles.

Page 27, line 25, for apex read base.

Page 29, line 18 from below, for apical antennal bristles read apical bristles.

Page 34, line 13 from below, for O. silantiewi read 2. O. Silantiewi.

Page 40, line 3, for parts read pairs; line 11 from below, for eye-shaped read eye egg-shaped.

Page 42, lines 7 and 8, for margin composed of read margin of. Page 45, line 10 from below, for segments.—Apex read segments: male.—Apex.

page 46, line 23, for concave above read convex above.

Page 49, line 7 from below, for third read longest apical bristle of third.

Page 50, line 10, for 8s read 7s.

Page 57, line 3 from below, for 8t read F. elata botis differs from F. elata elata as follows: 8t.

Page 58, line 6, for 7s resembling read 7s of E. elata botis resembling.

Page 65, Fig. 63 should be inverted; Fig. 66, the labels 8s and 8t should be interchanged.

Page 66, line 6 from below, for fourth read sixth.

Page 72, line 15, for carved read caved; line 11, from below, for Text figs. 77 and 78, read Text figs. 77 and 83.

Page 73, for Fig. 78. Rectofrontia tenella, female. (After Jordan.) read Fig. 83. Rectofrontia insolita, female. (After Jordan.).

Page 75, line 1, for Text figs. 82 and 83, read Text figs. 82 and 78; for Fig. 83. Rectofrontia insolita, female. (After Jordan.) read Fig. 78. Rectofrontia tenella, female. (After Jordan.).

Page 77, line 24, for Genal composed read Genal comb composed.

Page 79, Fig. 89 should be inverted.

Page 82, line 3, for male read female, not pigmented in male.

Page 91, Fig. 111, for the label pat read par.

Page 98, line 18 from below, for four paratypes read four in paratypes.

Page 107, the corresponding line of *Rectofrontia jaonis* (Jord.), in the last column, *for* Do. *read* Shansi; the corresponding line of *Nycteridopsylla galba* Dampf, in the last column, add Soochow after Shanghai.

Page 108, the corresponding line of *Citellus dahuricus mongolicus*, in the second column, for *Diamanus mandarinus* (J. and R.) read *Ceratophyllus tesquorum mongolicus* J. et R.; add Mongolia after Shensi in the last column.

Page 111, line 2 from below, last column, for Mongolia read Shanghai; line 1, from below, last column, for Shanghai read Mongolia.

Page 112, add an asterisk before 15. DAMPF.

Page 113, add an asterisk before 31. HICKS; also before 37. IOFF.

Page 114, add an asterisk before 54. JORDAN; also before 62. JORDAN.

Page 115, add an asterisk before 68. JORDAN; also before 69. JORDAN, before 75. LIU, and before 77. LIU.

Page 116, add an asterisk before 100. Rothschild.

Page 117, add an asterisk before 115. Wagner; also before 118. Wagner.

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[New names and new combinations are printed in beldface.]

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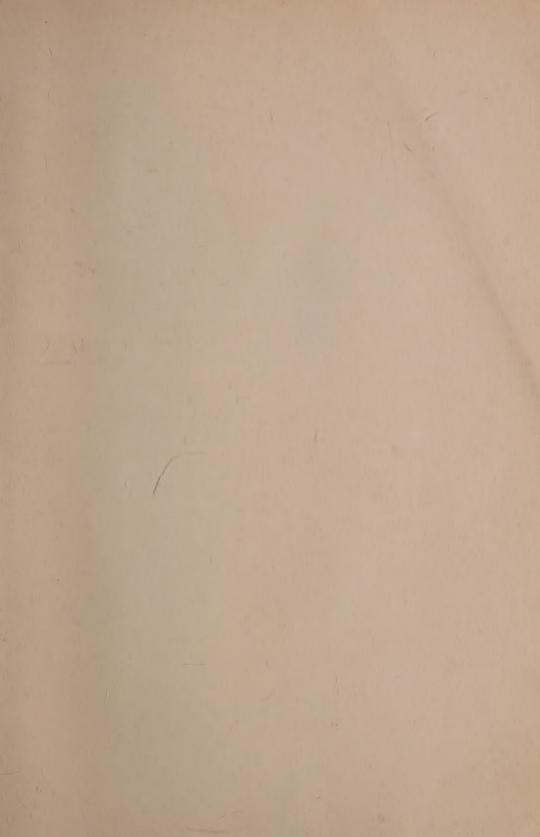
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